

No hemlock in future harvest

The US National Academy of Sciences has missed a trick in failing to make its white paper on genetic manipulation into an effective attack on present bumbledom.

THE US National Academy of Sciences has done a valuable public service by deciding to speak some simple common sense about the release of genetically-engineered organisms to the environment. Whether it will be much thanked for having done so is another matter. For what an academy committee under Dr Arthur Kelman, the Wisconsin botanist, has said is that enough is now known about the nature of genetically engineered organisms, and about the relationship between them and other kinds of artificial varieties (animal as well as plants), for the consequences of what is called controlled release to be predictable. There are many other than Mr Jeremy Rifkin, the activist, to whom this conclusion will be unpalatable. Those whose profession is the maintenance of regulations, and even their elaboration, will naturally be downcast. It is more worrying that there persists, in spite of fifteen years of restraint on research (some of it voluntary), a general unease outside the technical community at the potential, if not the practice, of genetic manipulation. How well will the academy's committee have put that to rest?

By the academy's idiom, the occasion is important. Like most other learned societies, the US academy does not easily arrive at collective opinions on matters of general importance. Although a prolific publisher of pronouncements on matters as different as containing the spread of AIDS and the problems of engineering education, these opinions are not put out as collective views, although great attention is paid to the basis on which the arguments are founded and the logic by which the academy arrives at its conclusions. The Kelman document was intended to be a little more than the standard publications, a policy statement ranking with what governments call white papers. What will happen to it now that it has been leaked to the *New York Times* at the weekend is anybody's guess.

Survival

The Kelman report nevertheless says all the right things. The observation that all possible organisms are likely already to have had at least a fleeting existence in the course of evolution is not a valid defence of the release of anything and everything to the environment; for one thing, the assertion is probably incorrect, but randomly generated organisms are unlikely to have occurred in circumstances that favour their survival. On the other hand, "accumulated experience in plant and animal breeding" of the conventional kind shows that artificially bred organisms are at a disadvantage in the 'wild', suggesting that artificially engineered organisms are competitively disadvantageous. If anything, genetic manipulation of the DNA of an organism will be safer than conventional breeding because of its precision — unwanted genes with unpredictable effects are not, for example, carried along — so that the 'new' organisms that people wish to introduce into the environment are strictly analogous to the varieties and cultivars that the conventional breeders introduce (and for which they are now able to secure breeders' rights).

The weakness of the document is that it continues in this vein of generality. What of the chance that genetically engineered crop plants will turn out also to be superior weeds, as in H.G.Wells's splendid *Food of the Gods*? The Kelman report correctly observes that for a plant to be successful as a weed, it

requires several of a considerable constellation of genetic attributes with which it is unlikely to be endowed by deliberate manipulation in the laboratory. The committee is right to say that plant breeders using the new techniques will have no incentive to provide their creations with mechanisms for the wide dispersal of their seed or with the means of making sure that seeds remain viable for long periods and germinate quickly when the environment is favourable, or any other of the mechanisms by which plants spread without financial advantage to their creators. But this will not satisfy the protesters, who will remark that circumstances may be very different when laboratories all over the world are practising these techniques. And what, they will ask, will ensure that malevolent experiments are not mounted, perhaps by military agencies who find it convenient to consider that the existing conventions prohibiting biological warfare do not interdict warfare against other people's crops?

Havoc

The academy's white paper is on more politic ground when it argues the need that those who plan experiments and succeeding commercial developments should at the outset give careful consideration to the biological characteristics of their organisms and the nature of the environment into which they will be introduced. After all, there is now a considerable catalogue of occasions on which the deliberate introduction of exotic but naturally occurring species into natural habitats has caused havoc. The committee is right to distinguish between engineered plants used in the artificial and reasonably well-understood conditions of modern farming, where it is even possible to engineer methods of control, and the introduction of engineered species into natural environments whose complexity may not yet be unravelled. So, the Kelman committee says, "the scientific community urgently needs to provide guidelines to both investigators and regulators in evaluating planned introductions of modified organisms from an ecological perspective". That would be handy. But is not the danger that the regulators will be the providers, and that the scientific community will have to live within the guidelines, no matter how unpalatable or nonsensical?

That is why it would have been an even greater public service if the academy's white paper had dealt directly with the regulatory issues that now unreasonably impede research and development in the genetic manipulation of plants (where quarantine is often mechanically impossible). It is of course ridiculous (as the committee says with more gentility) that artificially bred varieties should be dealt with differently according to the manner in which they are produced, but equally there is every chance that public confidence in the potential boon of DNA manipulation will be the more quickly established if the likely effects of introducing novel varieties are deliberately assessed in advance, not merely by the manipulators but by some detached body of invigilators. The intolerable feature of present arrangements in the United States is the opportunity, apparently endless, for unreasoned dissent to obstruct benign development. It is no wonder, if nothing to be proud about, that some investigators from time to time kick over the traces (see page 659). □



Going slow on AIDS

The US General Accounting Office says AIDS must be tackled more energetically.

THE general belief that the Reagan administration is not sufficiently energetic in its attempts to control the spread of AIDS (acquired immune deficiency syndrome) was last week confirmed by an unexpected source — the agency of government called the General Accounting Office (GAO). Asked on 16 June by the chairman of the Senate subcommittee responsible for appropriations to the Public Health Service for an opinion of the administration's budget proposals, GAO has done what many reporters have been doing these past months, but with commendable thoroughness and speed.

GAO has talked to people familiar with the disease either professionally or as participants in several studies of the consequences of AIDS for the US population that have appeared since last year's joint study by the US Academies of Medicine and of Science (*Confronting AIDS*, see *Nature* 324 3; 1986). GAO officials have visited cities where AIDS is a serious problem, talking to local public health officials. They have also held discussions with activist groups as well as the Public Health Service itself. All this has been done in just two months. GAO's formal report was delivered on 12 August.

In general, GAO found support for the administration's primary prevention plans — containing the spread of human immunodeficiency virus (HIV) among intravenous drug abusers, educating high-risk groups and the general public, and expanding voluntary testing and counselling. But the investigation also uncovered a consensus that funds for these purposes are far from adequate. Worse, GAO says that the general perception of a lack of federal leadership on AIDS is more worrying than the lack of money.

In the best tradition, GAO has also firmly stepped outside its terms of reference to evaluate the administration's plans and has suggested less costly ways of achieving some of the administration's professed goals. Thus the report suggests that posters on public transportation would be a cheaper and more effective way to spread AIDS education than mailing information to people's home addresses. But GAO's main conclusion is that already reached by many of those familiar with the dangers of AIDS for the public health: education should be the first line of defence against spread of the disease. That was also the clearest line running through *Confronting AIDS*.

The Reagan administration and the Public Health Service have not yet taken this message to heart. Assistant Secretary of Health Robert Windom, head of the Public Health Service, contends that the government is getting its message across through the news media and in commercial advertising time donated by broadcasters for public service announcements. But the free time is unlikely to be prime time, and it is not the job of the news media to put out government propaganda, no matter how noble its object. Moreover, the administration's budget request for AIDS education, \$155 million for all activities, does not go far in the hard-sell advertising world.

It is also disingenuous, if not downright irresponsible, for Windom to suggest that the United States government has less need than foreign governments, such as those of the United Kingdom and of Sweden, to warn its people about the dangers of AIDS simply because the cost of treating AIDS patients is not a direct charge on the federal budget. In the United States, health insurance companies will naturally want to be partners in public education campaigns, as they have begun to be in New York state. But can Windom seriously be suggesting that the government abdicate its responsibility for the nation's health to the good will and public spirit of private companies? If so, he is making a mockery of his post as head of the public health service, and undermining the work of his surgeon general and others who are fighting hard to stop the spread of AIDS.

The administration's lassitude in the prevention of the spread of AIDS has many roots. Last year's well-publicized row between the Surgeon-General and the Secretary of Education, Mr William J. Bennett, may have blown over, but the administration remains shy of giving people explicit advice about their sexual behaviour. A more subtle explanation of its diffidence on AIDS may be that it is trapped by the American conviction that problems arise only so that they can be fixed, preferably by action. Preventive medicine is embodied in the running shoes that impel a large proportion of the population along their local highways every day (sometimes with consequences that are the opposite of those intended), so if AIDS is as grave a threat to the long-term future as the evidence suggests it could be, should it not be met with decisive action of some kind? Can education, slow and necessarily uncertain, be a sufficient remedy? Patient perseverance, even when it is the only policy, is always a tough row for the White House to hoe. When President Reagan and his cohorts in the White House were frustrated by the delay in freeing US hostages in Beirut, they took action even though it was a bizarre contradiction of their declared policy of not bargaining with hostage-takers; are they not likely to be seduced by the notion that victory against AIDS will be won by armies of researchers armed with the mighty weapons of molecular biology rather than by the slow process of modifying behaviour, mostly sexual? What the US government needs to do is somehow to persuade its constituents that there will be no quick win for AIDS, but a lifetime's need for care and vigilance. And the sooner it begins, the sooner it will begin to dent the problem. □

A monsoon failure

This year's failure of the Indian summer monsoon is not as tragic as it once would have been.

THERE are two views of the failure of this year's summer monsoon in India. One is that the unhappy outcome of this year's seasonal roulette is but a familiar part of India's history. Inevitably, the years in which the June skies do not burst are years of tragedy. As always, India's poor will suffer most, especially in those regions not yet transformed by the tube-well and the green revolution, but there will be hardship even in the prosperous Punjab. More probably, many in India will be confirmed in the old fatalistic belief that they can do nothing against the great seasonal catastrophes, of which the failure of the monsoon is merely the most serious in the calendar.

That is the bad news. The good news is that India has been able to build up a stock of 23 million tonnes of grain over the past years of plenty, perhaps a third of annual consumption. With good management and the likelihood that irrigated areas will still produce a substantial part of their yield in a good year, India should be able to avoid, with its own resources, the widespread famines that followed the past failures of the monsoon. Much will depend on the care with which the available grain can be shared out among those most in need of it; corruption and the selfishness of state governments will be a nuisance. India may also emerge as a buyer on the world's grain markets, but need not on this occasion be a beggar.

There are several lessons to be learned. In India, where the prime minister, Mr Rajiv Gandhi, is the chairman of the committee set up to deal with the emergency, the government will rightly conclude that, for the long run, it had better follow even more energetically than in the past its policies for making India fertile. People in India and elsewhere who believe that the green revolution is simply a ramp for turning honest peasants into greedy farmers will at least be given pause. Other countries in which near-famine is almost a way of life may be persuaded to rethink their policies on agriculture. It is even possible that a monsoon failure that does not lead to utter catastrophe may serve to exorcise India's incubus of superstition, born of the conviction that there are no deals to be made with nature. □

Veterans' risk from dioxin "impossible to assess"

- Damaging health effects not found
- Potential medical costs a political concern

Washington

A LONG-AWAITED study of the health consequences for Vietnam veterans of exposure to herbicides contaminated by dioxin cannot be carried out in a scientifically valid fashion. That conclusion, certain to cause a political uproar, is based on a pilot study* by the Centers for Disease Control (CDC) in Atlanta that found no differences in dioxin levels in serum between ground troops in Vietnam and matched controls with no history of exposure.

From the time Congress required the dioxin study in 1979 it has been plagued by troubles. The Veterans Administration (VA) was first asked to perform the study, but veterans groups feared that the VA might not be impartial. If a study showed that dioxin — a contaminant of the herbicide Agent Orange — caused long-term health problems, the VA would have to foot the medical bills. So, in 1983, CDC took over the project, with the congressional Office of Technology Assessment (OTA) given the responsibility of approving the CDC study protocol.

At first, CDC thought its biggest problem would be to find enough veterans who had not been exposed to dioxin. In fact the opposite turned out to be true. It was very hard to quantify exposure based on records of troop movements, making comparisons between 'high' and 'low' exposure groups virtually meaningless. Nevertheless, CDC was prepared to start interviewing subjects and conducting health examinations at the beginning of 1986. But OTA balked, not satisfied that CDC had resolved the exposure issue. CDC director James Mason warned that even four months' delay could cost \$1 million, but the work was put on hold while CDC conducted a pilot study to provide a quantifiable indication of dioxin exposure (see *Nature* 320, 476; 1986).

Clinical chemists, using a technique called high-resolution gas chromatography/high resolution phase spectrometry, developed an accurate method of determining dioxin levels in serum. According to Vernon Houk, director of the Center for Environmental Health at CDC, the test can measure levels as low as 3 parts in 10^{12} . CDC tested the blood of 665 Vietnam veterans, divided into three groups based on exposure ratings from troop movements. They found no significant differences between the groups nor between those who said they had health problems and those who were in good

health. Even more discouraging, the median for all veterans tested for dioxin was 3.8 parts in 10^9 — the median for a matched control group of veterans who did not go to Vietnam was 3.9 parts in 10^9 .

There is no question that dioxin was a contaminant of the Agent Orange used in Vietnam, and that it can be detected in serum long after exposure. The Air Force has been studying troops involved in Operation Ranch Hand, the code name for the defoliant-spraying activities. Ranch Hand veterans tested this year still show serum dioxin levels above 100 parts in 10^9 . Comparing frozen serum drawn in 1982 with repeat sampling done this year, CDC now believes that the half-life of dioxin in serum is about 7 years. As defoliant spraying occurred in the late 1960s, significant exposure should still be measurable today.

Doubts also remain about dioxin's effect on humans. Although its toxicity in animals is known, the adverse health effects in humans have yet to be conclusively demonstrated. An Air Force study of Ranch Hand veterans has failed to show any increased mortality or morbidity among those known to have had significant exposure to dioxin.

The decision to halt the Agent Orange Study may come next month. The Agent Orange Working Group science panel reviewed CDC's pilot study at a meeting on 6 August and, according to former panel chairman Carl Keller, the CDC data seem to make a scientifically valid study impossible at present. The Agent Orange Working Group will probably make its recommendations next month to the White House Domestic Policy Council. OTA's Agent Orange advisory panel meets on 27 August, and indications are that it too will conclude that the study cannot proceed.

But veterans groups are not likely to be satisfied with the arguments that troops cannot be found who were exposed to dioxin. Barry Kasinitz of Vietnam Veterans of America remains convinced that Agent Orange has caused health problems for veterans, and that the current study might force the VA to pay for their treatment. "Congress won't accept a cursory study", says Kasinitz. If he is right, it will take political courage to say that a valid study cannot be done.

Joseph Palca

* Serum Dioxin in Vietnam-Era Veterans — Preliminary Report. Morbidity and Mortality Weekly Report, Vol. 36, No. 28, pp. 470-475 (1987).

No AIDS in Antarctica

Washington

ANYONE intending to spend the entire winter at any of the National Service Foundation-supported Antarctic stations must be tested for the presence of antibodies to HIV, the virus causing AIDS (acquired immune deficiency syndrome). Those testing positive will not be allowed to spend the winter. That policy was announced in a letter from Peter Wilkniss, director of the division of polar programmes. The new policy does not apply to summer-time investigators. It takes effect in the 1988 austral winter. J.P.

Scientists rehabilitated

London

THE Soviet Supreme Court has posthumously rehabilitated a group of agrarian scientists who perished in the repressions of the Stalin era. They include Aleksandr Chayanov, Nikolai Kondrat'ev, Aleksandr Chelintsev, Nikolai Makarov and Leonid Yurovskii. Most of them had worked during the 1920s as consultants to various state institutions. V.R.

New ACOST members

London

FOUR prominent figures from the UK academic community have joined the 15 members of what was the Advisory Council for Applied Research and Development to form the British government's new Advisory Council on Science and Technology (ACOST). Together with Sir John Collyear, chairman of the Department of Trade and Industry's Technology Requirements Board, the new members are: Sir Peter Swinnerton-Dyer, chairman of the University Grants Committee; Sir George Porter, president of the Royal Society; Professor Keith Peters of the Royal Postgraduate Medical School; and Professor Leonard Maunder of the University of Newcastle upon Tyne.

The council is due to meet in September, when it is expected that the future of the national space programme and continuing membership of the European Organization for Nuclear Research (CERN) will dominate proceedings. S.L.H.

Dirac medals awarded

London

THE 1987 Dirac Medals of the International Centre for Theoretical Physics (ICTP) at Trieste, Italy, have been awarded to Bruno Zumino, of the University of California, Berkeley, and Bryce de Witt, of the University of Texas, Austin. The awards were made in recognition of Zumino's leading work on field theory over the past 25 years and de Witt's fundamental contributions to the study of classical and quantum theory. Since 1985, ICTP has awarded the medals annually in memory of the late Paul Dirac. S.L.H.

California court forces closure of biology laboratory

San Francisco

LEGAL precedent was set earlier this month when the state Court of Appeal ordered research to be halted at the pharmacology school of the University of California at San Francisco. It is believed to be the first time that a court has shut down a major university facility to allay public fears over the dangers of research in molecular biology.

The action is the second of two unexpected defeats for the university in a long-running battle with a neighbourhood association opposing the opening of a laboratory at Laurel Heights in San Francisco. In July the Court of Appeal ruled that the university's environmental impact report was inadequate. In August this was followed by an injunction to close the laboratory for 90 days. Only rapid action by the state Supreme Court saved the research programme from destruction.

Two days into the shutdown the court granted the university temporary emergency relief from the injunction but followed this with a decision barring the laboratory from using radioisotopes.

The Court of Appeal's decision stunned university researchers. The environmental impact report prepared for public scrutiny had cited detailed evidence that the research was no different from that carried out at molecular biology laboratories elsewhere and was not dangerous to the public. Among that evidence was data collected at the university's main campus showing that there was no increase in levels of toxic or radioactive chemicals in areas near similar laboratories. That has since been backed up with a study commissioned from the Radian Corporation showing that, in the worst case, exposure of the local community to Laurel Heights laboratory emissions

"would be from 600 times to 500 million times less than levels known to be safe." The studies have not persuaded local residents. Accusations have been made that one of the researchers, Dr Nina Agabian, is involved in "biological warfare research". Dr Agabian is in fact a molecular parasitologist studying vaccines that may be of value in the third world. The university has repeatedly stressed that it does not do classified research.

The university's attorney, Etan Schulman, believes that local residents' response "may reflect a fear of technology and the unknown that is perhaps understandable in the light of the Chernobyl accident". Others are less charitable and perceive a deliberate campaign of misinformation by those who oppose any university expansion in residential areas. Either way, Mr Schulman is concerned that the courts may now be putting an unrealistic burden of proof on the university. In ruling the environmental impact report inadequate the court seemed to suggest that the rule of "substantial evidence" should be replaced by an impossible demand that the university must prove that their activities could never produce any harmful effect.

To restart its plans, the university must now win state Supreme Court appeals on both the injunction and the adequacy of the environmental impact report. The court has 60 days to decide whether to hear the appeals or simply to let the lower court decision stand.

The dispute already appears to have damaged the university. Several cell biologists have sought work elsewhere rather than risk delay. But the university is not necessarily blameless. In acquiring the Laurel Heights site it said that the research to be conducted there would be "academic" — a term more redolent of the library than the laboratory. Only much later did it start to speak of "academic and scientific research". With many universities anxious to expand urban campuses the need for skilled public relations is sure to grow.

Alun Anderson

A new future for Israeli defence research in the balance?

Rehovot

THE fate of the new Lavi fighter remains in the balance after a vote to decide the controversial fighter's future at this week's Israeli cabinet meeting was postponed. Lavi, a project that would propel Israel into the big leagues of military hardware production, has been sharply criticized both within the country and by the United States as project costs rose a long way above expected levels. But the debate over Lavi points up the need seen by many to bring about drastic changes in Israeli defense research, given the country's current economic situation.

Dr Zeev Bonen, outgoing director general of RAFAEL, the Israel weapons development authority, says projects such as Lavi, the Merhava tank and the Saar missile boat are simply too costly for a country the size of Israel to undertake on its own. Bonen favours multinational development cheaper weapons systems.

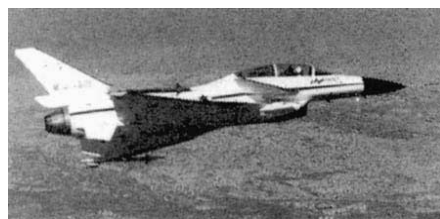
As director of RAFAEL from 1970 to 1978 and again from 1982 until last month, Bonen was at the centre of the accelerated defence research and development effort that began after French President Charles de Gaulle embargoed all arms shipments to Israel following the Six Day War in 1967. But the push for independence in arms "had a major weakness", says Bonen.

It was carried out with "a complete disregard of the economic imperative". Demand in Israel for weapons produced domestically "has almost always been far

below the break-even point in economic terms", says Bonen. Exporting weapons has been difficult, because many countries have political objections to buying Israeli arms, and competition in arms sales is fierce.

But multinational collaboration has its own difficulties. Because Israel will almost always be a junior partner in joint projects, there is a legitimate fear that Israeli science-based industry would be denied a significant role in the research, development and production process.

Maintaining adequate levels of security is also a problem in cooperative ventures.



Lavi — too costly for Israel alone?

"Secrecy norms in the West are completely different from ours", says Bonen.

Many in Israel — including those in the armed forces — agree that projects such as Lavi are too ambitious for a small country to undertake. For two decades, the push for total self-sufficiency in arms has guided Israeli military research, development and production, says Bonen. Despite the difficulties, he believes economic and political reality dictates that this policy must change.

Nechemia Meyers

East German base now independent?

London

EAST Germany is to have its own research station in the Antarctic. For some years, the East German Academy of Sciences has maintained an Antarctic research programme at the Soviet Novolazarevskaya station. On 1 July, however, the East German "Georg Foster" base became fully independent, and the first solely East German expedition will set out in October with a research programme including meteorology, geophysics, geology, atmospheric physics, isotope research, environmental studies and biology.

Vera Rich

Kinetic-kill weapon doubts

San Francisco

KINETIC-KILL vehicles, the tiny homing rockets some members of the Reagan administration are counting on for early deployment of a ballistic missile defence, will have a tough time hitting new solid-fuel and quick-burning Soviet SS-24 and SS-25 missiles, a new study indicates.

The latest scepticism over 'smart rocks' rises from analysts deep in the ranks of the Star Warriors at the Lawrence Livermore National Laboratory in California.

The conclusions are likely to complicate efforts to convince Congress to allow an early deployment of a missile defence system. They emerged from an analysis published in Livermore monthly magazine *Energy and Technology Review* by Christopher T. Cunningham, Philip Duffy and Thomas Morgan.

The Livermore trio devised a computer model that simulates the acceleration, speed and accuracy of kinetic-kill vehicles, or KKV's, deployed from polar-orbiting satellites 500 km up. The Livermore simulation gives the defence 20 seconds to spot ballistic missiles rising through the troposphere, and assumes KKV's can achieve 20g acceleration and a velocity of 6 km per second with a 90 per cent chance of hitting the target. Against today's ponderous liquid fuelled SS-18 and SS-19 missiles, the KKV's "may be effective", the study concluded. The currently deployed Soviet arsenal of 7,000 strategic missile warheads could be 75 per cent destroyed by an armada of 10,000 KKV's, and might be stopped entirely by two or three times that many.

However, a solid-fuelled SS-25 is another bird altogether. With its motor burning for just 3 minutes, and a single warhead to deploy, it would be hittable by a satellite-based KKV for just 3.8 minutes.

Worse for the KKV scheme, the Soviets could alter their rocket nozzles for a faster burn, and deploy their multiple warheads all at once rather than one by one, cutting the time a KKV has to reach the target. "We are very sceptical of the wisdom of relying on the first generation of kinetic kill vehicles to provide any real protection", Cunningham said.

In the heavily politicized atmosphere surrounding the Strategic Defence Initiative, no opinion can be considered disinterested. The Livermore laboratory is one of the bulwarks of research into successful missile shields, but its main job is to refine high-energy lasers, and other exotic weapons — programmes some scientists there see as jeopardized by any expensive, crash programme for early deployment of simpler systems.

Charles Petit

Museum officials confident *Archaeopteryx* is genuine . . .

London

OFFICIALS of the British Museum (Natural History) this week announced the results of new tests which they say prove that the museum's fossil specimen of the primordial bird *Archaeopteryx* is not a forgery. The announcement coincides with the opening of an exhibition at the museum, whose subject is the allegations by Sir Fred Hoyle and others (see below) that the feather impressions on the fossil were forged in the nineteenth century.

The museum staff have used ultraviolet light to reveal which parts of the fossil contain significant amounts of organic material — organic matter fluoresces under ultraviolet light. Only the remains of the bones fluoresce (see figure). Hoyle and the others have alleged that the feather impressions — around the bones of the wings and tail — were added by pressing modern feathers into wet cement laid over a genuine fossil of a featherless reptile. That the feather impressions fluoresce

under ultraviolet no more strongly than the surrounding bare rock shows that they are not on a layer of the sort of cement claimed to have been found on material from the slab — ground-up limestone mixed with organic glue.

Dr Angela Milner, curator of fossil birds at the museum, stressed that the new data was only the most recent of several lines of evidence that support the authenticity of the fossil. Dr Alan Charig said museum staff strongly resented allegations of dishonesty and are denying the doubters access to the specimen.

The exhibition sets out to present both sides of the forgery controversy. The centrepiece is a closely guarded display of the museum's *Archaeopteryx*, together with casts or photographs of the other five known specimens. Although the museum has made it clear that it believes that the fossil is genuine, visitors to the exhibition are challenged to study the evidence and make up their own minds. Giles Courtice

. . . but opponents renew demands for proof

THOSE trying to prove that the *Archaeopteryx* fossil is a fake have already placed on record their belief that the exhibition at the museum is unfair to their case. They have also made renewed demands to be allowed to test more material from the fossil.

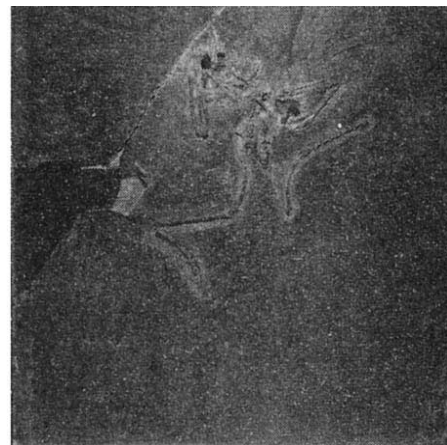
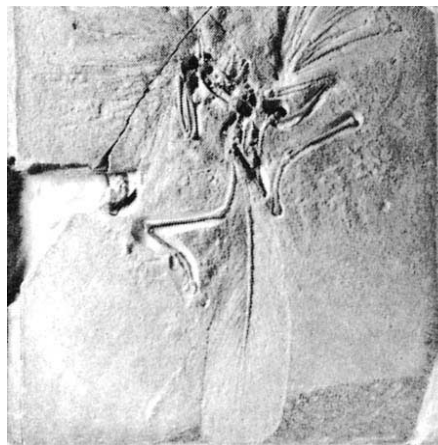
The group, which includes Professors Fred Hoyle and Chandra Wickramasinghe, both astronomers from University College, Cardiff, and Professor Lee Spetner, an Israeli physicist, claim that the chemical tests they propose could quickly settle the matter — using minimal amounts of rock. But the museum says all relevant tests have already been carried out, and that none casts doubt on the fossil's authenticity.

In 1986, Spetner was supplied with material, from parts of the fossil separate from the main area of feather impressions. He claims that scanning electron microscopy (SEM) and X-ray spectroscopy tests

revealed differences between the parts of the fossil bearing feather impressions and other parts of the stone slab (see *Nature* 324, 622; 1986). If the feather impressions were added in the nineteenth century to a real, featherless fossil, such a difference might be expected. But the museum says it is probable that one of the samples happened to include traces of a substance put on the fossil to preserve it.

The forgery theory, spelt out in a recent book by Hoyle and Wickramasinghe (see *Nature* 324, 185; 1986), has received little support from biologists, palaeontologists or geologists. But Hoyle sees the museum's refusal to supply the doubters with more material as evidence of an "unscientific" unwillingness to settle the issue. The museum refuses to risk damage to the specimen for tests they consider unnecessary.

Giles Courtice



Archaeopteryx (ultraviolet picture on right) — early bird or tarred and feathered reptile?

High-speed train for Australia bites the million-dollar bullet

Sydney

A MULTI-MILLION dollar feasibility study of a 350 km-an-hour high-speed rail-line linking Sydney, Melbourne and Canberra was announced earlier this month by a venture company formed to conduct the project.

First indications that the project would proceed came from New South Wales Premier Barry Unsworth during a trip to Japan. Unsworth met representatives of Kumigai-gumi, a Japanese construction company with a high profile in Australia and one of the partners in the train venture. Kumigai is supplying the knowhow for a A\$450-million tunnel running under the Sydney harbour. After a ride on the Bullet Train, Unsworth's enthusiasm ran away with him. He not only expressed his support for the project, but added, much to the surprise of the private companies that will pay for the new rail line, that a full-scale feasibility study would proceed.

Known as the Very Fast Train (VFT) and travelling faster than any train existing, it would cover the 876 km between Sydney and Melbourne in three hours. The VFT route takes it around the seaward side of the Snowy Mountains, with the exception of Canberra a fertile but sleepy rural corner of the continent. The present rail link goes inland, to the west of

the mountains, missing Canberra. The trip from Sydney to Melbourne takes 12 hours.

VFT is the brainchild of Dr Paul Wild, who started his career as a radio-astronomer and retired as chairman of the Commonwealth Scientific and Industrial Research Organization in 1985. He says VFT should take five years to construct at a cost of A\$4,000 million. He hopes to see it finished in 1995.

According to Wild, the speed of the train will be exploited to reduce construction costs. Because the kinetic energy of a body in motion increases as the square of its velocity, the very high speed of the train can be tapped to carry it over hills regardless of the gradient. This allows the use of much steeper gradients than conventional trains can cope with, perhaps up to 1 in 20, and greatly reduces the need for massive earthworks.

The economic viability of the VFT depends on the passenger market, an issue to be explored in the first stage of the feasibility study. A A\$600,000 pre-feasibility study gave very positive results, suggesting the likelihood of more than 3 million trips a year. This number would be accommodated by about 30 trains running each way daily. According to Wild, as a rule of thumb, each person living

along the rail catchment area makes one trip a year. Almost half of Australia's 17 million population lives in the Sydney-Melbourne area.

Besides Kumigai, the other partners in the project are an Australian-based transport multinational, TNT Management, with interests in road and air transport, and Elders-IXL, a Melbourne wheelbarrow-maker which has grown into a diversified multinational.

TNT is cutting its teeth on rail transport with the construction of a monorail around Sydney's central business district which is due for completion in time for Australia's bicentennial next year.

Charles Morgan

Canada and Ireland promote science ties

Dublin

LIKE Canada, Ireland is seeking to foster closer ties between industry and its research community, as well as encouraging companies, particularly small ones, to develop research expertise. To this end, Dr Sean McCarthy, Minister for Science and Technology, has recently launched a new IR£1-million science and technology programme.

Although the ministry's programme will emphasize work in electronics and engineering — including IR£1 million for equipment to the Institute for Industrial Research Standards — there is also IR£0.6 million for the establishment of a national biotechnology programme. This money will be used to set up three centres of excellence at third-level colleges. These will be used to generate new products and processes as well as acting as sources of trained personnel for industry. The centres will focus their efforts on diagnostics, cell culturing and food technology.

The IR£2.5 million is in addition to the IR£14 million allocated in the annual budget to the various state-funded science and technology bodies. Although the additional research funding is relatively small, it is nonetheless welcome in the current climate of budgetary cutbacks. Announcing the new programme, the Taoiseach (Prime Minister) Charles Haughey undertook to make it a permanent feature of the annual budget. An expanded second phase of the biotechnology programme is already being planned for next year.

McCarthy's appointment as science and technology minister following the Fianna Fail electoral victory earlier this year marks the first time there has been a minister with specific responsibilities for science. The science and technology ministry is a junior ministry within the Department of Industry and Commerce.

Mary Mulvihill

Canadian science and technology to make a happy marriage?

Toronto

In an attempt to bring off a happy marriage between advanced science and technology and Canadian industry, Prime Minister Brian Mulroney's government has announced the creation of a new national Department of Industry, Science and Technology. The new department will cover the entire country through three regional industrial development offices. Although the budget for the whole department has not been made public, the office in Edmonton, Alberta, will have a budget in excess of C\$1,000 million.

The new department seems to have been inspired by the Japanese Ministry of International Trade and Industry. Until now, Canadian research and its main agencies, including the National Research Council, have reported to Parliament through a policy-making ministry with no real spending power or clout. The new department brings Canada into line with many other industrial nations by tying science to industry.

The department merges the present Ministry of State for Science and Tech-

nology with the Department of Regional Industrial Expansion. It will be headed by Industry Minister Michel Côté with Frank Oberle, the current Science Minister, as his junior.

The new department is intended to reflect Canada's commitment to a technologically advanced industrial economy. Part of its role will be to help establish new knowledge-based industries to improve the country's long-term international competitiveness.

There is uncertainty about how the new department will work. Professor Alan Carswell of York University, a past president of the Canadian Association of Physicists and president of Toronto-based Optech Inc., does not want to judge the new department too hastily. "The words describing it are reasonable", says Carswell, but he adds that many Canadian scientists are disillusioned by cuts in research budgets. Carswell also says the government scheme to have industry provide matching funds for research has been a heavy burden for industry.

Russell McNeil

US fears of Soviet satellite getting ahead

London

THE Soviet Earth-sensing satellite, Kosmos-1870, launched on 25 July, is causing some concern in the United States. Although the official Soviet announcements treated it as just one more satellite carrying out remote-sensing work in fields that include hydrology, cartography, geology, agriculture and the study of the environment, US space officials see it as a further indication that the Soviet Union is building up a considerable lead in space technology.

They see the K-1870 as an analogue of their own Earth Observing System, (EOS) — with the difference that K-1870 is now in orbit and EOS will not be ready for launch until the mid-1990s. Furthermore, at an estimated 20 tonnes, K-1870 is some 7–10 times heavier than either the most advanced US Earth resources satellites now operational (Landsats 4 and 5) or the largest civilian Earth resources satellite ever launched. Even the premature re-entry last week of the polar-orbit reconnaissance satellite Kosmos-1871 has failed to dent the image of Soviet prowess evoked by K-1870.

The official Soviet announcements followed the routine low-key pattern typical of Kosmos launches. They did, however, stress that the satellite was launched by a Proton launcher (the services of which are now available commercially to countries seeking a launch facility for their satellites) and especially stressed K-1870's radar facilities.

From the very beginning, the Soviet space programme has placed considerable emphasis on remote sensing "in the interests of the national economy", and although this catchphrase may in the early days have been intended in part to allay public disquiet about the cost of the programme, it has more than justified itself by results not only in weather forecasting but also in the location of mineral and water resources.

Remote sensing is playing an increasing part in 'international' missions conducted under the Interkosmos programme. The programme of the recent Soviet-Syrian mission included photographic and spectrometric surveys of Syria, aimed at geological and hydrological prospecting, the study of agroindustrial resources, and investigation of atmospheric and coastal pollution. Furthermore, the Soviet Union is now inaugurating a commercial space-based photographic survey service, offering a better resolution than any similar service that is available commercially in the West.

Vera Rich

French company use Chinese satellite

Paris

THE use of a Chinese satellite to carry out French microgravity experiments last week was "completely independent of the temporary suspension of Europe's Ariane rocket launches", according to a spokesman from Matra, the company behind the experiments. It was the "friendly relationship" between Matra and the Chinese government that led to the offer of space on the Long March 2 rocket. Matra, which builds the vehicle-equipment bays for Ariane rockets, is to supply electronic equipment for the Jiuquan launch site in western China.

Two experiments were carried out, an accelerometry test of gravitational forces and a study, using algae, prepared by Matra in association with a bioengineering laboratory of the *Centre National de la Recherche Scientifique*. The collaboration was agreed at June's Paris Air Show, the satellite was launched on 5 August and the data were received in Paris 10 days later. Matra believes this is the first time a western industrial company has collaborated with the Chinese government on a space project and expects to repeat the exercise in the future.

Peter Coles

Unauthorized release upsets EPA

Washington

IN a move that he characterized as "civil disobedience", a Montana State University researcher admitted last week that he had released a genetically engineered microbe into the environment without the approval of the US Environmental Protection Agency (EPA). Plant pathologist Gary Strobel said he inoculated 14 elm trees with a recombinant strain of *Pseudomonas syringae*, whose enhanced antifungal activity he had developed to combat Dutch Elm disease.

Strobel injected the trees with the recombinant bacteria on 13 June, two days before he applied for EPA approval for the field test. Strobel said he was not aware of the regulations that require the EPA to clear all releases of genetically engineered organisms into the environment until a colleague warned him. If he had waited for approval, he said, he would have been forced to delay the test until next season, and lose a year's work.

Strobel's action highlights the frustration of many researchers over the lengthy review for field tests, but he could now be denied government funding permanently or face criminal charges.

Carol Ezzell

World's longest bridge network for Japan

Tokyo

THE world's longest network of bridges linking mainland Japan (Honshu) to the island of Shikoku was completed last week.

The Seto Ohashi (big bridge), which spans nearly 10 km by straddling five islands in the Seto Inland Sea, is a massive feat of engineering that has engaged more

than 2,000 construction companies over the past nine years and has cost ¥1.13 million million (\$7,500 million). There are three suspension bridges, two cable-stayed, one truss and five viaduct bridges forming a 'double decker' — the bridges have a dual carriageway for cars and will have a railway underneath.

Two more multi-bridge systems linking Shikoku and Honshu are under construction. To the east of Seto Ohashi, a road/rail link between Kobe (on Honshu) and Naruto (Shikoku) is due to be completed in 1998 and will include the world's longest suspension bridge, with a central span of nearly 2 km, which, like Seto Ohashi, will be a double-decker. To the west, construction of a 9-bridge road link between Onomichi (Honshu) and Imabari (Shikoku) has been under way since 1975. Two bridges are complete, two are under construction, and, in line with Japan's policy of boosting domestic demand, construction of three more should begin next year.

Seto Ohashi will open to road and rail traffic in April next year, allowing the crossing to Shikoku to be made in 10–20 minutes instead of at least an hour by ferry. But the saving in time comes at a high cost. To repay the enormous sums borrowed for construction, the Honshu-Shikoku Bridge Authority is expected to set the toll at about ¥5,000 (\$33) one way.

David Swinbanks

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Honshu-Shikoku Bridge Authority

World's longest bridge and highest toll?

Stanford looks to the west

Palo Alto

STANFORD University is about to become the first US university directly to tap Japan's strengths in scientific and technological innovation. Following the signing of an agreement with Kyoto University at the end of last year, plans are now being completed for the construction of a centre in Kyoto that will allow US science and engineering students to carry out some of their undergraduate and graduate studies in Japanese industrial companies.

Stanford has benefited enormously from close links between its science and engineering schools and new high-technology industries, in particular the semi-conductor and computer industries of Silicon Valley and the emerging biotechnology industries in the San Francisco region. Both are industries with good reason to fear Japanese competition — or to welcome cooperation. What the future will hold may, according to the proposal for the new centre, depend in part on correcting the 'asymmetry' in the US relation with Japan and producing "Americans who are familiar with Japanese institutions".

The aim is not to produce a new generation of japanologists. That job is to be done in a separate Center for Japanese Studies which Stanford University is setting up in Kyoto with six other US universities. There, students and researchers specializing in Japan studies can find a home. The Stanford Center in Technology and Innovation (SCTI) has a different goal, one that stems from the view that people knowledgeable about Japan are coming from too narrow a base of economic and professional skills. The plan is to take 30 engineering and science students a year, give them 6 months part-time training in "survival Japanese", then send them to Kyoto. With the cooperation of Kyoto University staff they will spend 3 months learning about Japan, with emphasis on the study of technology and innovation and their impact on industrial societies and international trade. Following that will be 3 months of hands-on experience at a Japanese industrial or government institution. The hope is that the contacts and experiences gained will stand the United States in good stead when these people go on to become tomorrow's policy makers, computer engineers and biotechnologists.

Beyond its classroom programme, SCTI will also accommodate researchers and try to build a reputation as a multi-disciplinary centre for the study of technology and innovation. Some contribution to the US-Japan trade imbalance may come early on: funds to build the new centre are being raised by donations from Japanese industry.

Alun Anderson

India carries out large-scale tests of anti-leprosy vaccine

New Delhi

INDIA entered the race to develop an anti-leprosy vaccine last February when large-scale clinical trials of the indigenously developed ICRC vaccine began in the state of Maharashtra. Already, 2,500 healthy household contacts of leprosy patients have been vaccinated, and the figure will reach 40,000 by 1990.

The vaccine was developed in 1979 by Dr Madhav Deo and his colleagues at the Cancer Research Institute (CRI), in cooperation with the Acworth Leprosy Hospital, G.S. Medical College and the Haffkine Institute, all in Bombay. The project is financed by the Indian Council of Medical Research in New Delhi.

The vaccine contains gamma-ray-inactivated ICRC bacilli, named after the Indian Cancer Research Centre (ICRC), the previous name of CRI. The bacilli are a group of leprosy-derived slow-growing mycobacteria that have been cultivated repeatedly from human lepromatous for the past 30 years. Although the exact taxonomical position of the ICRC bacilli is somewhat uncertain, they supposedly belong to the *Mycobacterium avium* intracellular complex. The fact that the bacilli exhibit antigenic cross-reactivity with *M. leprae*, especially with reference to T-cell immunity, the dominant host defence against the leprosy germs, forms the scientific basis for the use of ICRC bacilli in the preparation of an anti-leprosy vaccine.

Since 1979, the vaccine has been administered in phase 1 and phase 2 clinical trials to patients with lepromatous leprosy and to healthy subjects in an endemic area. The vaccine enhanced immunity in both patients and healthy subjects. It was cleared for large-scale clinical trial by the Drug Controller of India for both immunotherapy and immunoprophylaxis in October 1984.

A pilot project conducted during the past three years on about 200 healthy household contacts of leprosy patients shows that the vaccine has no untoward side-effects. Fears that lepromin-positive healthy contacts may develop hypersensitivity to leprosy antigens resulting in nerve damage have been set to rest.

The objective of the large-scale trials is to make a comparative evaluation of the immunoprophylactic efficacy of the two vaccines containing either ICRC bacilli or BCG by measuring the incidence of all forms of leprosy in the vaccinated groups. To achieve statistically significant data, 40,000 people will be vaccinated.

Elsewhere in the world, two other large-scale clinical trials have been launched in the past three years using vaccines containing *M. leprae* from arma-

dillo alone or in combination with BCG. The trials in Venezuela are supported by the World Health Organization, while those in Malawi are supported by LEPRO in the United Kingdom.

"Because ICRC anti-leprosy vaccine contains cultivable mycobacteria, it is not only cheap but carries no risk of contamination with animal tissue products", says Deo. The ICRC anti-leprosy vaccine has high acceptability because it is given as a single dose. On the other hand, Deo says, the vaccines containing armadillo-derived *M. leprae* are costly but also contain foreign (armadillo) proteins and DNA, and unknown retroviruses that may produce delayed harmful effects.

The Maharashtra trial is the first ever large-scale clinical trial of a vaccine containing cultivable leprosy-derived mycobacteria. The vaccine is important for India, which accounts for one-third of the 12 million cases of leprosy world-wide.

K.S. Jayaraman

BTG's income falls

London

INCOME of the British Technology Group (BTG), a state-owned management company set up to assist the commercialization of innovative ideas, fell 23 per cent last year to £14.89 million, compared with the 1985-86 figure of £19.35 million. The net surplus for the year, after tax, was £1.99 million, compared with £5.77 million for 1985-86.

Describing the year as "one of change and consolidation", BTG's annual report claims that its final figures were much as expected, despite two major hiccups during the year: a sharp reduction in income from pyrethrin insecticides because of unforeseen market fluctuations in China, and a slowing down of revenue from royalties on magnetic resonance imaging (MRI) machinery pending legal action against Johnson and Johnson in the United States, which was settled out of court in December. MRI will now become the money spinner for BTG — in June an agreement was signed with General Electric for MRI licensing rights, which included a down-payment thought to be of the order of millions of dollars.

Inventions received from the public sector were up from 355 to 388, although the number of industrial projects, at 192, is at its lowest "for several years", which BTG blames on improved availability for financing from other sources and the "relatively low profile which BTG has taken in the industrial finance area over the past few years". Simon Hadlington

British sheep still contaminated by Chernobyl fallout

London

HUNDREDS of thousands of sheep in Britain are still contaminated by radioactive fallout from the Chernobyl nuclear reactor explosion. Although most other countries have removed restrictions, the British government last week introduced new measures controlling the movement and slaughter of sheep in Wales and Scotland on farms previously designated as safe; restrictions are also still in force in Cumbria in northern England.

Caesium washed into the peaty, waterlogged soil by heavy rainfall after the nuclear plant accident has remained accessible to plant roots, with little clay or loam to bind up the radionuclides. The new season's grass and other pasture plants have taken up caesium and effectively concentrated it, so rough-grazing sheep have ingested quantities of radioactivity. More than 500,000 sheep on 564 hill farms are now under restrictions, according to the Ministry of Agriculture, compared to the four million sheep involved in the initial emergency restrictions imposed in June last year.

Experiments are being conducted by the Institute of Terrestrial Ecology into the effects on caesium uptake of applying

a clay mineral, such as bentonite. Controlled field experiments have shown that when bentonite is spread onto pasture and consumed by grazing animals, it can absorb caesium in the gut of sheep or cattle. There are, however, practical difficulties in applying bentonite in the high-caesium areas, where grazing animals roam over miles of open fell country. Techniques of hand-feeding bentonite as a slurry are also being studied.

Ministry of Agriculture officials confess to being somewhat surprised at the continuing high levels of caesium contamination in the soil and the pasture vegetation. The problem could persist for years, according to Dr John Curtis, head of a ministry team studying the effects of Chernobyl on food and agriculture.

Monitoring of humans in affected areas has shown extremely low levels of radiation, according to the National Radiological Protection Board (NRPB). Results showed levels of caesium 134 and 137 of 25 microsieverts in highly affected areas, compared to 10 microsieverts in the unaffected area of Oxfordshire.

The limit of radioactivity for sheep being used by government inspectors is 1,000 becquerels per kilogram, in spite of

the opinion of some scientists that 9,000 becquerels per kilogram, the limit originally recommended by NRPB, would be more appropriate. The lower limit is in line with other European Economic Community (EEC) countries.

The EEC is considering new radioactivity limits to replace the Chernobyl emergency limits which expire in October. The European Commission had recommended caesium limits of 1,000 becquerels per kilogram for dairy products, 1,250 for other foodstuffs, 800 for drinking water and 2,500 for animal feedstocks. Other recommended limits for dairy products are 500 for isotopes of iodine and Strontium, and 20 for isotopes of plutonium and other alpha-emitting nuclides; for other foodstuffs, 3,000 for iodine and strontium, 80 for plutonium; and for drinking water, 400 for iodine and strontium, 10 for plutonium.

Kathy Johnston

UK looks East at Chernobyl

London

ENGINEERS of the UK Atomic Energy Authority (AEA) have devised the name "positive scram" to describe the sequence of events that put paid to one of the nuclear reactors at Chernobyl in the Ukraine a year ago, but think it unlikely that there will be a recurrence.

The British post-mortem on Chernobyl, discreetly made public on the eve of the anniversary of last year's debriefing conference at Vienna, is apparently one of several conducted by authorities in countries such as the United States, Canada and France. "Scram" is the name for the process of shutting down a reactor in a hurry, and "positive scram" that for the circumstances in which emergency action has an effect opposite to that intended.

Atomic Energy of Canada Ltd seems to have taken much of the initiative in deciding what went wrong last year. The root cause of the accident, it seems to be agreed, is a sequence of errors and illegal procedures so surprising that the British AEA believes they may have been habitual. The net effect was that the reactor was operating in a condition far from normal, in which the excess reactivity of a thermal reactor derives from the 0.7 per cent of all neutrons originating from fission daughters of uranium-235 — with a delay of some seconds.

The decision of the operators at Chernobyl to withdraw one batch of control rods beyond their normal range is also reckoned to have been a serious contributory cause of the accident. But John Gittus of the AEA believes that the changes made to Soviet reactors since the accident will have sufficed to make plants of that kind safe.

Philip Campbell

"Another Chernobyl" in the Ukraine?

London

A LEADING Ukrainian writer, Oles' Honchar, has called for action to stop what he describes as a "second Chernobyl". In radio and press interviews celebrating the rehabilitation of his controversial novel of the 1960s, *The Cathedral*, Honchar spoke forcefully about a "collective letter" just issued by citizens of Cherkassy Oblast', outlining a classic example of bureaucratic confusion and bad planning. It was decided, he said, to build a coal-fired power station on the Dnieper, but then it was found that transporting the coal to the chosen site would not be cost-effective. So the station was redesigned to run on fuel-oil, piped in from the Kremenchug refinery. Only after the outlay of vast sums of money was it realized that the oil would not flow in the pipelines in the winter, so the station was then redesigned for nuclear power. This, Honchar said, was in spite of the fact that the area is densely populated and there were no proper guarantees given against pollution of the Dnieper, which is the principal source of water for the southern Ukrainian industrial belt.

There is considerable opposition to the scheme from scientists, Honchar said, and the official go-ahead for the plans has not yet been given. Nevertheless, the All-Union

Nuclear Power Construction Unit is already "frantically" at work, transferring the population of several villages and tearing down architectural and historical relics. The site of the proposed station is Chyhyryn, the capital of the independent Ukrainian Cossack state of the seventeenth and eighteenth centuries, an area that is virtually an open-air museum "where



Chyhyryn, by Taras Shevchenko (1814–61).

every step echoes the history of the people", and where many of the relics scheduled for demolition were portrayed in the writings and paintings of the Ukrainian national poet, Taras Shevchenko. Honchar's protests and criticisms have now been supported and amplified by other Ukrainian writers, including the dissident Vasyl Zakenarchenko. Vera Rich

An ethical dilemma

SIR—Since 1985 it has become apparent that an important correlate of successful infection with the AIDS (acquired immune deficiency syndrome) virus is an interaction of virus genes with the lymphocyte genes regulating the switch in growth (G0/G1 transition) necessary for resting lymphocytes to enter the cell cycle.

Optimal progress in medical research depends on communication and collaboration between researchers who, at the same time, are competing with each other for increasingly scarce research funds. If the collaboration between two researchers is symmetrical ('tit-for-tat'), then both researchers should benefit and medical research should progress optimally. But perfect symmetry is hard to attain. Thus, a researcher becomes aware that collaboration may help another researcher who may then gain an advantage. This communication/competition paradox may be starkly evident in the case of researchers who, while trying to optimize progress towards the cure of AIDS, are also trying to optimize their own research funding.

In the summer of 1985, after studying the lymphocyte growth switch for more than 20 years, I reported the successful application of a novel cloning strategy to the first preparation of a human lymphocyte cDNA library likely to be enriched in G0/G1 switch genes¹. I was not surprised to be approached shortly afterwards by several laboratories engaged in AIDS-related research (I.C.Y. Chen, UCLA; W.A. Haseltine, Harvard; D. Baltimore, MIT), with requests for samples of my recombinants. I responded promptly to these requests. With small grants from the American Foundation for AIDS Research and the Leukaemia Research Fund (Toronto) I continued to isolate and characterize more recombinants from the library. In the intervening two years, no papers reporting the preparation of a similar library from lymphocytes have appeared. This also is not surprising because success in library preparation requires the coordination of specialized skills both in the culture of human lymphocytes and in recombinant DNA technology.

Now we come to the ethical dilemma. I recently submitted grant applications to three major funding organizations (NIH, MRC-Canada, NCI-Canada). In all of these I included the following sentence: "In view of the seriousness of the AIDS epidemic, it would have been most unethical to have delayed distributing our recombinants while awaiting funds for their study in our own laboratory."

All three applications were rejected. The following major criticism by the committee which reviewed one of the applications (NCI-Canada) is, I believe, not

untypical of the attitude of many who sit on such committees: "It was noted that the applicant had already sent his potentially interesting clones to several large laboratories. There was concern that much of the interesting work would be done by these laboratories instead of by the applicant himself."

So there we have it. Altruistic conduct in research, even in the case of a matter so serious as AIDS, does not appear to be appreciated by the hard-nosed individuals who sit on grant review committees. I now have many more recombinants at a stage where they can be distributed to others in the AIDS-research community. Have I learned my lesson? Do I hold on to them and refuse to respond to future requests for samples? How many other researchers have valuable recombinants which they are not prepared to make available to other AIDS researchers?

In my case there is no ethical dilemma. The new recombinants (unpatented) will be sent promptly and without charge to assist any relevant investigation in medical research. The matter, however, raises the larger issue encapsulated in the phrase, "the communication/competition paradox". Have the organizations funding medical research seriously considered suggestions for modifying their procedures in order to reduce the intensity of competition and encourage communication and collaboration between researchers? The modern system of research funding, established immediately after the Second World War, has been very successful. But the positive role of competition in this success has been questioned². Would progress in medical research have been even more spectacular if research funding were organized differently? It has been argued that the present system promotes patterns of conduct among researchers that impede progress^{3,4}. Numerous suggestions for reforms have been made^{3,6}. But there are few indications that the funding bodies can be shifted from their complacency. Meanwhile, the number of deaths from AIDS continues to rise exponentially⁷. The cost of the management of just one AIDS patient (approximately \$100,000) would keep an average AIDS research laboratory operational for a whole year.

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Japanese whaling

SIR—David Swinbanks' account of Japanese concern about the ban on whaling is well-balanced¹ but one aspect was not mentioned.

Small-scale whalers in Japan operating in coastal waters argue that they have the same "aboriginal subsistence" whaling culture as do Eskimos and that the International Whaling Commission should exempt them from the imposed moratorium². To me, the logic of this seems far-fetched for at least the following reasons.

(1) Although whale meat is an important component in the daily diet of Eskimos³, it at present constitutes only 2 per cent of animal protein in the daily intake of Japanese according to the Japan Whaling Association⁴.

(2) Whereas the Eskimos are still maintaining their traditional method of catching whales by an open whale boat (called *umiak*) consisting of a wooden frame covered with hide and propelled with broad paddles, the Japanese transformed their traditional methods of net whaling to industrial whaling in 1899^{5,6}.

(3) For Eskimos living in the Arctic environment, which is limited in numbers of natural flora and fauna, whaling is a necessity for survival. But this necessity does not now exist for Japanese. Whaling would have been a necessity in past centuries when Japanese refrained from eating other kinds of meat. But contemporary industrialized Japanese society is hardly dependent on whale meat for survival.

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Litigious challenge

SIR—I noticed with interest the recent contributions on the possible connection between cooking in aluminium vessels and the onset of Alzheimer's disease.

It will be interesting to see what happens when the first American lawyer realizes that Alzheimer's patients may have been taking aluminium in quite large quantities in antacid preparations.

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Chemists come in from the cold

New developments in understanding high-temperature superconductivity have given theoretical chemistry a new lease of life, which Linus Pauling has been quick to recognise.

HIGH-TEMPERATURE superconductivity has at least two considerable achievements to its credit already. Predictably, perhaps, there has been a renewed flurry of excitement in solid-state physics. People seem to be measuring anything they can, and not always with one of the ternary copper oxides as the substrate. Sensibly, the several oxides of copper are favourites, but anything with a layered or, ideally, a perovskite structure is a natural. Even if the temperature of bulk superconductivity is never increased above 90 K or so, surely something must come out of all that.

But the most remarkable of the transformations of the past few months is that chemists have once again been welcome in august society. (That should not be taken to imply that chemists have ever ceased to be powerful; the present president of the Royal Society of London, for example, like two out of the three physical scientists who previously held that post, is a chemist.) They, after all, are the people who know about atomic radii, bond lengths, valency, coordination numbers and electronegativity, all of them concepts a little more shadowy than when they are first written on the schoolroom blackboard. In passing, these same people may help with telling how to put one's hands on a little ytterbium, or gadolinium, if the competition should become even rougher.

It is nevertheless amusing to witness the way in which hardliners in the field, people who know about the Meissner effect and how to measure it, appear now to be willing to take instruction on chemists' topics such as valence-bond theory from people they would not ordinarily (or even now) trust to calibrate an electrometer. They even let Linus Pauling read them a lesson on just that subject the other week in *Physical Review Letters* (59, 225; 1987). But even Pauling seems not quite sure whether resonating valence bonds will account for high-temperature superconductivity; there is a splendid giveaway phrase in his paper, a conjunction that goes, "Whatever may be the mechanism of superconductivity, it is certain that..." Can there ever have been clearer proof that people are at a loss?

True superconductivists may therefore be comforted to know that valence-bond theory has not always been popular, even among chemists. Pauling's own great book (with A.H. Wilson), *The theory of the chemical bond*, now more than half a century old, had the curious effect of turn-

ing disciples into detractors. It was for many people a splendid way of learning wave-mechanics, but it turned out also to be a demonstration that precious little could be calculated; only supercomputers have materially changed that.

Forty years ago, the name of everybody's game was to calculate the properties of systems with more than one atom using the wavefunctions of hydrogen, or of atoms like it with a nuclear charge that might be different from unity. The valence-bond route required one to enumerate all possible ways in which the available electrons might be distributed among the available nuclei, however unphysical they might seem. The valence-bond calculation of the hydrogen molecule ion as a resonating mixture of two equivalent states of a free proton and an intact hydrogen atom (the electron can be interchanged) was obviously a kind of a joke with a serious message (because it can be made to work).

How to get better wavefunctions? Then, as now, people were at sixes and sevens. Linear Combinations of Atomic Orbitals (LCAO) were convenient, in the sense of being easy to construct, calculate and fit to the few known facts by the adjustment of a few parameters. The obvious snags were the adjustable parameters and, more philosophically, the circumstance that the molecular orbitals that, for example, made it a fact of life that the outer electrons of a molecule such as benzene are utterly delocalized, did not comprise a strictly orthogonal set, which is what people had been told was a necessary condition for using them. At the time, the chemists appear not to have appreciated that people like Léon Brillouin had made striking progress on a complicated version of a similar problem simply by representing the state of an electron in a metal as that of a particle in a box and using heuristic (or hand-waving) arguments to show that the result is bound to be a band structure of some kind.

Intrinsically, all three ways of calculating macroscopic systems should yield the same result if they were soundly based and were able to take account of enough terms in the inevitable perturbation series. Over a long interval, heroic work has been done by those who reckoned at an early stage that there would be benefits (for others, not often themselves) in finding wave equations representing electrons in the outer shells of complicated atoms. Now, it

is all so much easier. Those who use up-to-date atomic wavefunctions refer to them by four- or six-character alphanumeric codes and expect their readers to know precisely what they mean.

The physics of the problem has, unfortunately, not been much changed. The better the machines become, the larger the number of terms that can be included in approximations to the truth and, thus, the greater the difficulty in telling what they signify. In the end, it will not matter much; the approximations will converge. Meanwhile, there is this urgent problem of telling what explains the high transition temperature to superconductivity in the ternary copper oxides...

Pauling's answer is magisterial; he senses that the square arrays of oxygen atoms surrounding each copper atom in what is presumed to be the superconducting plane of the ternary oxides are the means by which pairs of electrons are assembled and then able to move together from one inter-copper space to another. In passing, he figures out that scandium should be better than barium as a temporary storage place for (or donor of) electrons in the active superconducting plane.

Other ruminations show common cause. For example, J. E. Hirsch, from the University of California at San Diego, building on an earlier suggestion by V.J. Emery, has shown how electrons may be held together physically in pairs (and thus made subject to Bose-Einstein condensation) when moving in an antiferromagnetic lattice by the penalty of having to break nearest-neighbour antiferromagnetic bonds as they go (*Phys. Rev. Lett.* 59, 228; 1987). The fun in that is that Hirsch appears not to know that his antiferromagnetic bonds (oppositely directed electron spins) are what Pauling calls valence bonds. It may be a smaller world than we think.

Meanwhile, the question will remain of whether it will be possible to calculate these structures: if the valence-bond method is so often hampered by the number of alternative structures that must be included in the basis set, will even Crays suffice? It is also strange that, so far, the chemists have been remarkably silent on a question that might be thought to interest them most — why should marked departure from stoichiometry matter so much? Perhaps there is still a lot to learn.

John Maddox

Human disease

A multiple sclerosis therapy?

Byron H. Waksman

In the 13 August issue of the *New England Journal of Medicine*¹, Dr Murray Bornstein and colleagues at the Albert Einstein College of Medicine (Bronx, New York) report significant improvement in patients with exacerbating–remitting multiple sclerosis receiving long-term therapy with Copolymer-1 (Cop-1) in a double-blind controlled study. Cop-1, a synthetic polypeptide analogue of the myelin basic protein (MBP) of central nervous system myelin, was administered daily over 2 years to 25 patients with mild–moderate disease who were experiencing 1–2 exacerbations (relapses) per year. The average exacerbation rate dropped to 0.3 per year, whereas in 23 control patients receiving a placebo on the same schedule, the rate was 1.3. Fourteen individuals in the treatment group and six in the placebo group remained free of exacerbations. In those with the mildest disease, there was less progression of disability with treatment than with placebo. The drug was relatively non-toxic, causing at most some redness and soreness at the injection site and, rarely, palpitation, flushing and breathing difficulties.

These results might seem promising, but they also raise questions. First, the local reaction at sites of Cop-1 injection could have compromised the blinding of individuals on the drug; this suggests the possibility of a placebo effect. Further, those with relatively mild disease did best on Cop-1. Although this is the group that might be most affected by any treatment, this is also the group most affected by the emotional and psychological factors that contribute to a placebo effect.

Approximately 150 'therapies' have been advocated for multiple sclerosis the past 50 years². Most current therapies are based on the rationale that this disease results from viral infection and/or autoimmunization against one or more components of myelin. The use of Cop-1 is the only recent attempt at *specific* immunotherapy. It follows naturally from the observation, first made in the 1950s, that the model disease, experimental autoimmune encephalomyelitis (EAE), is prevented or arrested by injections of myelin components such as MBP in animals. One unsuccessful attempt³ has been made so far to arrest the multiple sclerosis disease process by similar injections of MBP. Cop-1 shows immunological cross-reactivity with MBP and also prevents EAE, so it was appropriate to test it in human subjects. But batches of Cop-1 used in the present study¹ were found to vary markedly in their ability to suppress EAE.

Most current attempts to arrest the process of multiple sclerosis involve *nonspecific* antiviral, immunosuppressive or immunomodulatory agents⁴. The table lists the best known of these as well as newer, more sophisticated, specific and nonspecific approaches to immunotherapy⁵. It is important to distinguish these therapies from therapies directed at the inflammatory process, that is, the tissue expression of the disease process (among these are adrenocorticotrophic hormone, adrenal steroids, colchicine, gold compounds and calcium chelators); functional changes resulting from the lesions (such as 4-aminopyridine on conduction, amantadine on fatigue and other agents on pain, tremor and bladder function); and the many 'therapies' (from hyperbaric oxygen to special diets to snake venom) advocated on empirical grounds. Adrenocorticotrophic hormone and high-dose steroids, such as methylprednisolone, appear to exert a palliative influence during acute attacks of multiple sclerosis. On the other hand, toxic agents such as high-dose cyclophosphamide appear to arrest the disease process, but for no more than a year or two. Alpha and beta interferons are reported to reduce the frequency of exacerbations, perhaps by reducing the number and intensity of viral infections. As Cop-1 is an effective interferon inducer, its effect may be nonspecific.

The problem of evaluating a new therapy for multiple sclerosis is complicated in two respects. First, the course of the disease is unpredictable and patients show a strong placebo effect. In a brief controlled trial of adrenocorticotrophic hormone some years ago, 60 per cent of the controls given placebo showed significant clinical improvement after a few weeks of 'treatment'. Therefore, a strict double-blind format, with stratification of patients by

sex, age, severity and type of disease, and statistical randomization of treatment and control groups is obligatory⁶. The efficacy of blinding is a key element determining the validity of the trial.

The second problem concerns the evaluation of the ongoing disease process. Those affected by the disease, as well as members of their families and the larger community, are principally concerned with the subjective and objective manifestations of the disease and the degree of associated handicap. However, recent studies make it abundantly clear that there is a gross discrepancy between overt clinical symptoms and signs and the actual lesions in the central nervous system, of greater interest for scientific understanding and the attempt to design better therapies⁷. Therefore, various laboratory techniques have been brought to bear to evaluate the number and size of lesions⁸. Magnetic resonance imaging (MRI) is the most powerful of these; its value is enhanced by physiological methods that demonstrate lesions interrupting long sensory or motor tracts (visual and other evoked potentials, magnetic brain stimulation). Separate tests also permit the evaluation of local vascular injury and fluid leakage (enhanced CT-scanning, gadolinium MRI), the immune response against myelin antigens (free and bound antibody against MBP), the nonspecific immune response (oligoclonal bands of immunoglobulin G and free κ -chains in cerebrospinal fluid), the local inflammatory process (macrophage activation in blood and spinal fluid), and the rate of tissue breakdown in lesions (free peptides of MBP in spinal fluid, blood and urine). Little of this very expensive technology has yet made its way into current practice in therapeutic trials, as exemplified in the present instance¹.

The official position of the Medical Advisory Board of the National Multiple Sclerosis Society of the United States is that, although Cop-1 may offer hope for reduction of frequency of exacerbations in people with mild multiple sclerosis, a further multicentre clinical study is

Therapeutic attempts to arrest the disease process in multiple sclerosis

Therapies tested in recent past

Antiviral agents: interferons and interferon inducers, acyclovir, ribavirin.

Myelin-specific immunological tolerance: MBP, Copolymer-1.

Non-specific immunosuppression and immunomodulation: cyclophosphamide, azathioprine, cyclosporine, total lymphoid irradiation, plasmapheresis, heparin, thymus hormones, transfer factor, anti-lymphocyte antibodies.

Therapies to be tested in near future

Inactivated 'effector T cells' cloned from CSF: idiotype tolerance.

Active 'suppressor T cells' cloned from blood to CSF: antigen or idiotype tolerance.

Receptors of such cells of monoclonal antibody versus specific receptors: block specific immunological reaction and/or induce tolerance.

Monoclonal antibody versus 'non-polymorphic' recognition molecules (HLA-DR2, IFN- γ or its receptor, IL-2 receptor): block reaction and/or induce long-term suppression.

CSF, cerebrospinal fluid; HLA-DR2, major histocompatibility complex antigen of class II; IFN, interferon; IL-2, interleukin-2.

required to confirm the initial results and address questions such as those raised here. Cop-1 is not available for general use; it remains an investigative agent. Future supplies of a uniform, standardized Cop-1 preparation are not assured, and it is not clear when this agent will again become available for further testing. There are rumours of a 'black-market' in Cop-1. If so, it is important to recognize that there can be no assurance of source, quality, therapeutic effectiveness or safety with such preparations. □

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Cell motility

Good things from slime

T.J. Mitchison

DURING the past few years, visitors to the Schliwa-Euteneuer laboratory in the University of California at Berkeley have been entertained by the gradual spread of a large freshwater amoeba over the available bench space. This organism, which resembles a large piece of slime, first appeared on the sides of a fish-tank (see figure) and has since colonized large numbers of petri-dishes. Currently this amoeba has become the main research focus for members of the laboratory who are interested in the rapidly developing field of intracellular motility. This example of zoological opportunism seemed initially to be an amiable eccentricity, reminiscent of an earlier era of cell biology. But recent developments in understanding transport processes that occur in the amoeba demonstrate that a shrewd choice of experimental organism, however unprepossessing, can lead to important general insights, one of which Koonce, Schliwa and co-workers report on page 737 of this issue¹.

The amoeba (*Reticulomyxa*) grows as a large syncytium that can reach several centimetres in diameter. Like other syncytial organisms (*Allogromia*, *Physarum*), *Reticulomyxa* faces a more difficult problem than smaller cells in keeping different regions of its cytoplasm in constant communication with each other, and distributing food and waste materials appropriately. Similar problems are faced by highly asymmetrical cells in animals, notably neurons. *Reticulomyxa* appears to have coped by specializing its peripheral cytoplasm for microtubule-based

intracellular transport. The fine processes that radiate out from the centre of the organism are very active in bidirectional organelle transport along microtubules². The recent isolation of kinesin, a molecule capable of moving particles along microtubules³, has opened up such organelle movement along microtubules to molecular analysis.

Kinesin, an ATPase that 'walks' unidirectionally along the microtubule lattice, may drive anterograde transport in axons where all microtubules are aligned with their 'plus' ends away from the cell body^{4,5}.



A growth like this would prompt most people to clean out the fish tank. The syncytial amoeba, *Reticulomyxa*, growing on the side of an aquarium. The scale is in centimetres. (Courtesy of M. Schliwa.)

Another motor with quite different properties, mediating transport in the opposite direction, seems to exist in axons⁶. The motor for organelle transport in *Reticulomyxa* seems pharmacologically distinct from kinesin, and there are intriguing suggestions that, in contrast to axons, transport along the microtubule in opposite directions may involve the same or very similar motors, with directionality controlled by phosphorylation⁷. The development of an excellent lysed-cell system in *Reticulomyxa*⁸ should help

answer the important question of how transport direction is specified in the physiological context.

The fine processes in *Reticulomyxa* (see figure) are also very active in splaying out and zipping together, leading to a novel type of spreading motility. Generally, the movement of cells on their substratum is driven by actin-based systems, but *Reticulomyxa* lacks the fine actin-rich lamellipodia that most cells use to crawl, and its ultrastructure is dominated by microtubules. Previously, microtubules have usually been considered to participate in cell movement only by polymerizing at a leading edge (see, for example, ref. 9), but the splaying and zipping in *Reticulomyxa* seems much too rapid to involve new microtubule assembly. In their work reported in this issue¹, Koonce *et al.* demonstrate that bundled microtubules in *Reticulomyxa* processes can actively slide past each other *in vitro*, and they suggest that this sliding drives the motility of the processes *in vivo*. As the authors point out, sliding forces acting on tethered microtubules have long been known to drive ciliary beating, but theirs is the first demonstration of motility resulting from the active sliding of one microtubule past another, a process that could be involved in many other types of motility.

Although Koonce *et al.* have not yet identified the motor responsible for sliding, they exclude actin, and the pharmacology suggests a relationship to the motor that drives organelle transport. The bundles splay apart at both ends, and it is not clear which microtubules are bound to the motor, and which are being moved. Thus, the question of directionality of the sliding motor cannot yet be addressed as it has been in ciliary axonemes¹⁰.

If indeed the same, or very similar, motors do turn out to move organelles in both directions, as well as microtubules past one another, it will be extremely interesting to learn how these processes are discriminated and controlled. The authors are currently engaged in isolating microtubule-dependent motor proteins from *Reticulomyxa*, using the affinity of these proteins to microtubules. The best things from slime may be yet to come. □

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Palaeolithic archaeology

Pleistocene life in the dead heart of Australia

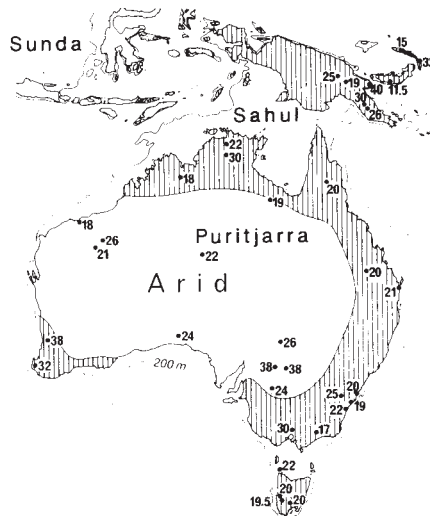
Rhys Jones

THE first secure date for the human occupation of Australia, extending back just beyond 10,000 years (10 kyr), was obtained only 25 years ago¹. The discoverer, John Mulvaney, referred to Australia as "the dark continent of prehistory". It is now known that humans occupied parts of Australia 40 kyr ago, a date close to the limits of the conventional radiocarbon method, and evidence of occupation extending back to 20–25 kyr ago has been established for almost all the main ecological zones of the continent (see figure). The one area that has stubbornly refused to yield such evidence beyond 10 kyr is the core of the arid zone, which encompasses about 60 per cent of the Australian land mass in its centre and west. Some believed² that the Australian desert was not occupied until the end of the last Ice Age, about 10–12 kyr ago, but that Pleistocene occupation was confined either to coastal regions or close to the banks of rivers and lakes, such as Lake Mungo, western New South Wales, which, although technically being within the arid zone, were fed by waters derived from areas with higher rainfall. Smith's demonstration, reported on page 710 of this issue³, of occupation 22 kyr ago at Puritjarra, a large rockshelter in a dunefield of the MacDonnell ranges north-west of Lake Eyre within the core of the Australian desert, finally refutes this view and, at a stroke, more than doubles the known antiquity of human occupancy of the region.

Whereas it is known that there were hominids in Java 0.7–1.0 Myr ago, there is no convincing evidence anywhere in the Indo-Malay archipelago or the adjacent mainland of South-East Asia (then joined as a contiguous Sundaland during glacial low-sea episodes) for stratified occupation sites, nor indeed for any coherent stone-tool assemblages significantly older than those already obtained from greater Australia^{4–6}. The archaeological data support the conclusion from work on mitochondrial DNA⁷ that biologically modern man could have used a new technology to penetrate the South-East Asian/Australian region 40–50 kyr ago. This process may have been analagous with contemporaneous events in western Asia and Europe, where Neanderthals were replaced by modern man⁸. In the east, it is probable that genetic interchange took place, leading to the continuation of a distinctive regional clade, as seen in Australian fossil crania⁹.

Jared Diamond, in a recent News and

Views article¹⁰, described how only small rodents managed to cross the 100-km-wide water barriers between Asian Sunda and Australian Sahul using natural means such as vegetation rafts. Humans must have used technology that included some kind of constructed craft. The first colonists must also have had an economic system based on the exploitation of some elements of tropical lowland rainforests and shorelines, so in what is present-day New Guinea and, to a lesser extent, in northern Australia, they would have experienced familiar conditions, with many species of edible plants, fish and



Oldest radiocarbon dates for human occupation of regions of Australia and New Guinea, expressed in kiloyears. The arid region of central Australia is defined as being within Prescott's climatic index (precipitation evaporation) 0.7–1.0. Pleistocene dates for New Ireland (top right) by J. Allen *et al.* (in preparation).

molluscs being common to both regions.

The earliest stone technology included large bifacial and waisted axe-like tools made of volcanic rock¹¹, found in New Guinea from the reef-edge coast to the highlands and in northern Australia. Groube has suggested¹² that these tools were used to ring-bark rainforest trees to open up the canopy and to stimulate the growth of edible weed species such as yams, sugar cane, bananas and tree fruits. Paradoxically, having mastered the capacity to cross water, the true barrier to the colonization of the Australian continent lay not in the occupation of the tropical north but in the extension south into the dry regions of the continent. Hence the significance of Smith's discovery³ of stone artefacts dating back to the Pleistocene.

Ethnographically, the main plant staples of the desert Aborigines were grass seeds such as *Panicum* sp. which were ground into a flour using sandstone grinding dishes. In central Australia, none of these dishes has been found before about 4 kyr ago¹². The original desert colonists may have had to rely on other plant foods such as the tubers of *Ipomoea*. There is growing evidence that late Pleistocene occupation of central arid regions of Australia was a general phenomenon, as exemplified by the basal dates of 21 and 26 kyr from two rock shelters in the Hamersley Plateau, Western Australia¹³; a hearth with fresh-water mussel shells of almost 14 kyr in the dunefields of the Strezlecki Desert¹⁴; and evidence of underground flint mining at 20 kyr in the arid Nullarbor Plain¹⁵. Occupation continued throughout the main arid phase of the last glacial maximum. This occupation increased markedly within the past 3–4 kyr^{16,17}, associated with the introduction of a stone-tool technology consisting of microblade and hafted flakes used as adzes, which itself might have been a reflection of other social transformations within prehistoric Aboriginal society.

The evidence found by Smith³ of red ochre associated with the 22-kyr date at Puritjarra strikes a piquant chord within the history of palaeolithic archaeology. It was Spencer and Gillen's demonstration at the end of the nineteenth century, that nomadic, stone-tool-using hunters of the desert of central Australia could also make the most profound artistic and religious creations, that prompted Salomon Reinach, the doyen of French archaeology, to propose a magico-religious function for the cave art of the Dordogne¹⁸. We now see that the distant ancestors of the Arunta Aborigines were painting at the same time as the Aurignacians, half a world away. □

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Solar physics

Models of motions in the Sun

Robert F. Howard

WITHIN the past 15 to 20 years there have been significant improvements in our ability to measure the line-of-sight motions at the solar surface with the Doppler effect in absorption lines of the solar spectrum. The resulting increased sensitivity has led to the detection of low-amplitude, more or less steady flows on the surface of the Sun. Superposed on the well-known differential (with latitude) rotation rate of the surface layers are very low amplitude, systematic motions — not the motions predicted by theoretical studies, but nevertheless observable, large-scale patterns that are related to the solar activity cycle. In their article on page 696 of this issue¹, Snodgrass and Wilson present a synthesis of the Doppler observations and other recent results in a model of convective rolls which, they suggest, is the subsurface velocity pattern that leads to these observed surface motions and that represents the fundamental mode of giant-cell convection in the Sun. This model may indeed represent the real physical situation in the Sun, but the issue is by no means resolved.

The nature of the solar activity cycle is yet to be described thoroughly in physical terms. Almost certainly the magnetic fields that are a common element for all cycle-related phenomena (such as sunspots) are generated beneath the solar surface in a dynamo-type process that depends on convection, differential rotation and perhaps other large-scale motions of the solar gas. Snodgrass and Wilson believe that the convective rolls which they postulate to exist are one of the important elements in the dynamo mechanism.

Convection, the transfer of energy by motions of a gas induced by temperature and density differences in a gravitational field, is one of the great unsolved problems in astrophysics. The effect is commonly seen, as in heated air rising over a warm radiator. In a star, convection is one of the principal modes of transferring energy from the interior, where energy is generated by nuclear reactions, to the surface where that energy is later radiated away. It is possible to describe in a simple fashion the gross properties of a convective gas, but a reliable modelling of the convective motions is still elusive. Theoretical studies of the onset of convection in a compressible fluid indicate that

very large cells — roughly the size of the depth of the convection zone — are efficient in transporting energy, and thus should be present in the Sun and other stars². These cells are expected to be elongated in the north-south direction, with boundaries coinciding with imaginary longitude lines on the Sun, but such 'banana-shaped' velocity features have never been found. The velocity limits set by the theoretical analyses are now in the neighbourhood of a few metres per second³, a very slow speed for an object with a circumference of roughly 9×10^9 m.

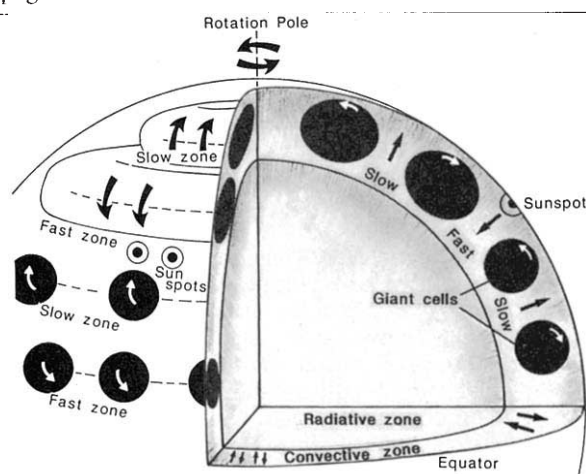
A different, unanticipated type of motion was discovered in the search for

to the generation of solar activity, it seemed reasonable to suppose that a zone of increased shear, represented by the boundary of the two velocity zones, would lead to the concentration of sunspots and other manifestations of solar activity.

This pattern of large-scale, low-amplitude motions was not predicted by any theoretical models. The people who discovered them thought that they represented a periodic torsional motion about the solar rotation axis — a motion that contributed to the cycle mechanism — and hence they were named 'torsional oscillations'. The restoring force for the motions was presumed to be a strong subsurface magnetic field. Very soon, however, an attractive alternative explanation was offered, and this explanation fits into existing dynamo models for the activity cycle. Yoshimura⁵ and Schüssler⁶ suggested independently that the motions at the surface represent a magnetic,

Lorentz force caused by the subsurface configuration of the magnetic fields thought to lead to the eruption of magnetic flux and to the formation of active regions. This model does not explain the existence of the velocity pattern at high latitudes, however, and in the absence of observational material or theoretical advances, the matter remained unresolved.

Ribes⁷ then performed a careful study of the motion of absorption features on the solar surface as seen in the H α line in the solar spectrum. These features are known to signal the presence of magnetic fields, so this is equivalent to tracing the motions of magnetic-field elements, and it is likely that this is equivalent to motions of the ionized solar gas at or slightly below the surface of the Sun. Ribes found that the motions of these structures, averaged over



Schematic cutaway view of the Sun showing the hypothesized convective rolls at high latitudes, breaking up into giant convective cells at low latitudes. The fast and slow torsional motions are shown. These zones migrate equatorwards during the course of an activity cycle, requiring 22 years to travel from pole to equator. Solar activity forms near one low-latitude shear zone.

these cells. Its pattern is characterized by zones divided by parallels of latitude that rotate alternately slightly faster or slightly slower than the average rotation rate for that latitude. In general, there are four such zones in the northern or southern hemisphere at any one time in a pattern symmetrical about the equator of the Sun. These zones, which have a velocity amplitude of about 6 m s^{-1} on top of the rotation rate (of $2,000 \text{ m s}^{-1}$ at the equator) migrate slowly towards the equator. The time required for a zone to migrate from the pole, where the zones originate, to the equator is about 22 years, or twice the length of a single activity cycle. The shear boundary between fast and slow zones (represented by a high-latitude slow zone and a low-latitude fast zone) is the locus, below about 30° latitude, for solar active regions. Because the differential (with latitude) rotation is believed to contribute

long intervals, could be represented by convective rolls stretched in longitude — that is, orthogonal to the banana rolls predicted by theory. Moreover, these rolls are seen to drift slowly polewards, opposite to the drift direction of the torsional oscillations. Thus, the description (let alone the explanation) of the large-scale motion field at the solar surface seemed to be in some disarray.

Shortly after that, Snodgrass analysed⁸ some of the synoptic velocity data from Mount Wilson that had not been previously examined and discovered that there are downwards motions associated with the torsional shear zones in the active-region latitudes. This suggested to Snodgrass and Wilson¹ that there is a large-scale pattern of convective rolls, somewhat similar to those discovered by Ribes, but migrating slowly towards the equator. The convective nature of the

pattern was suggested by the very small temperature variations with latitude discovered recently in limb scans that were designed to search for solar oblateness⁹. The velocity pattern itself may be suspect because there is a well-known effect that looks like a Doppler redshift of spectrum lines in magnetic-field elements on the solar surface¹⁰. Actually, it is probably an effect of the shape of the spectrum line resulting from a velocity or magnetic gradient with height in the solar atmosphere.

The consensus for now is a firmly established east-west, low-amplitude velocity pattern which is either a major part of the cause of the eruption of magnetic flux at the solar surface to make the solar cycle, or is a by-product of the subsurface magnetic-field configuration that makes up the cycle mechanism. At the same time there are long convection rolls that may be related to these torsional oscillations and drift towards the equator, or may be

totally unrelated to these phenomena and drift polewards. It does not seem likely to me that this confused picture can be clarified without either a significant addition to the observational base or a breakthrough in theoretical understanding of the dynamo mechanism and the large-scale convection in the Sun. □

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The *ras* gene

Transformer and transducer

Michael R. Hanley and Trevor Jackson

THE *ras* oncogenes and their protein products have an undisputed importance in the development of our present concepts of carcinogenesis. Indeed, clinical research recently reported in *Nature*^{1,2} shows that *ras* mutation may be causative or closely linked to the onset of some types of human tumours. Thus, it is surprising that, despite extensive work, the mechanism of action of *ras* is still unknown. Over the past year, there has been a flurry of reports addressing this question³⁻¹⁰, but the mystery remains as great as ever.

The products of the *ras* genes comprise a multigene family whose structural relationships are illustrated in the figure. Their amino-acid sequences all contain GTP-binding consensus regions, and they are thought to be localized to the inner surface of the plasma membrane¹¹, anchored by a covalent modification: palmitic acid attachment at a conserved cysteine in the carboxy terminus (see figure). The similarities between the *ras* proteins and the class of signal-transducing guanine nucleotide-binding (G) proteins have provided an appealing conceptual framework in which to pursue *ras* mechanisms. Thus, it has been a widely held prediction that the *ras* gene products would be components of a membrane-transduction process.

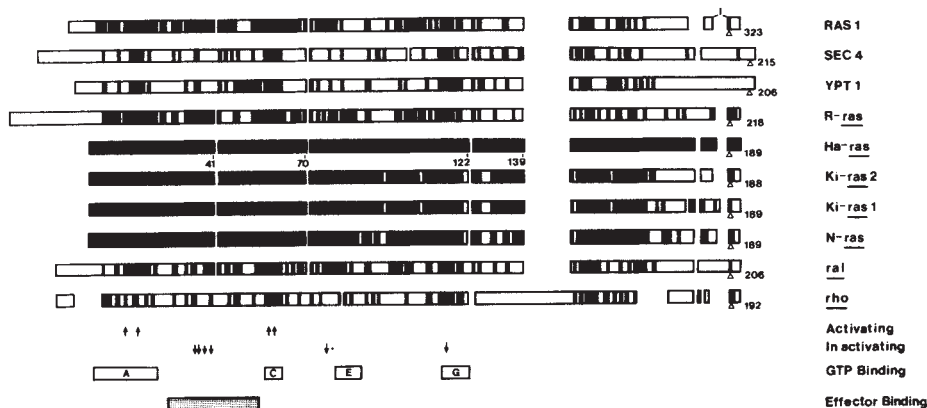
The first suggestion, by analogy with identified actions of similar proteins in yeast, was that the mammalian *ras* family would act as regulatory components in the activation of adenylate cyclase¹², but no such activity has ever been shown in

mammalian cells¹³. Thus, there was considerable interest in the possibility that *ras* species might act as the elusive G proteins of the inositol-lipid signalling pathways^{14,15}. The cellular analysis of this possibility was greatly assisted by the availability of a diverse range of *ras*-transfected mammalian cell lines. Induction of a steroid-hormone-regulated *ras* construction, causing overexpression of a cellular *ras* protein, causes amplification of bombesin-stimulated production of ino-

sitol phosphates³. However, more recent work from Racker's group shows that a similar inducible *ras* construction amplifies the inositol phosphate production stimulated by one growth factor, bradykinin, while it attenuates the action of another, platelet-derived growth factor⁴. Two other groups have reported^{5,16} that another receptor-linked second-messenger response, activation of adenyl cyclase, is blunted in *ras*-transformed cells. Thus, it is unlikely that *ras* species are functionally analogous to authentic coupling G proteins, but they nevertheless influence the efficacy of surface-receptor transduction pathways.

In all these circumstances, interpretation of the experimental results is complicated by the time required for *ras* induction, and the inevitable cause-and-effect problems of the transformed state. Indeed, induction of *ras* overexpression causes rapid (within 60 min), selective alterations in the activity of cellular genes, including a *sis*-related sequence that may encode a growth factor⁶. As Owen and Ostrowski correctly note⁶, "the time window in which *ras* action is monitored is important because the cellular milieu in which this gene operates is progressively altered". The generic problems of inducible constructions have therefore encouraged novel experimental designs that circumvent them.

Feramisco and his colleagues have pioneered acute microinjection of bacterially expressed *ras* proteins as a route to identifying the early events in *ras* action⁷. After *ras* injection, fibroblasts are induced to proliferate. Intriguingly, this approach can distinguish between transforming mutant *ras* species and normal proto-oncogene species in that the former elicit responses lasting for many hours,



Schematic comparison of deduced sequences for *ras*-related proteins. All sequences are compared with human cellular Harvey (Ha)-*ras*. Filled regions, exact identity with the Ha-*ras* sequence; breaks, non-conserved additions or deletions. The sequences, identified on the right, are from the following species: yeast (RAS1, SEC4, YPT1); human (R-*ras*, Ki-*ras*-1 and -2, N-*ras*); simian (*ral*); or *Aplysia* (*rho*). The number of residues in each sequence is indicated. Open triangles, conserved cysteine residues that correspond to palmitoylation sites; upward arrows, positions of transforming point mutations identified in Ha-, Ki- and N-*ras*. Downward arrows, point mutations which abolish transforming activity or activation of yeast adenyl cyclase. Boxed regions A, C, E, G correspond to previously named sequences that contribute to the GTP-binding site¹⁰. Hatched box, proposed sequence for effector interaction¹⁰; I, inserted sequence in RAS1 (not shown). These data summarize results from the original references (cited in refs 10, 22-24).

and the latter for less than 3 hours. The earliest changes are all events associated with the plasma membrane: ruffling, enhanced fluid-phase pinocytosis, and stimulation of phospholipase A₂ activity⁷. More recently, the advantages of microinjection of oocytes from the toad *Xenopus* have been exploited. Insulin promotes oocyte maturation, but two groups have now shown that broadly cross-reactive anti-*ras* antibodies can block the action of insulin on maturation^{8,9} and, further, that microinjection of oncogenic, but not normal, *ras* can induce maturation.

These new studies establish the important precedent that the insulin receptor tyrosine kinase can act on *ras*, thereby linking two significant problems: substrates of the insulin receptor kinase (discussed by J. Espinal in last week's News and Views¹⁷); and physiological regulation of *ras*. The insulin kinase may not be the only activator of *ras*. Earlier work by Stacey and his colleagues¹⁸ divided transforming kinases into two classes: those whose activity could be blocked by microinjection of anti-*ras* antibodies; and those whose transforming activity was unaffected by anti-*ras* injection. Intriguingly, those oncogenes that appeared to require the cooperation of cellular *ras* are all membrane-associated tyrosine kinases, such as *src*. As might be expected from these results, the epidermal growth factor receptor can also phosphorylate *ras*¹⁹.

A domain essential to transformation that is distinct from the GTP- and membrane-binding residues has recently been identified¹⁰, and may mediate effector interaction. If so, this domain is highly conserved in the mammalian *ras* species (see figure), implying that they all have a common effector target but differ in their mode of regulation, possibly responding to different kinases.

What could this common effector be? There is a novel non-proliferative output response to *ras* microinjection in PC12 cells, which differentiate from adrenal chromaffin-like cells into sympathetic neurons on nerve growth factor (NGF) treatment, where *ras* can induce NGF-like differentiation²⁰. Moreover, microinjection of anti-*ras* antibodies can block the NGF-induced differentiation²¹. Thus, it appears the *ras* effector is linked to differentiation, as well as mitogenesis, and can influence or trigger a range of cellular events.

One neglected possibility is to take the structural analogy with other GTP-binding proteins in a new direction. There are two yeast *ras*-like gene products, YPT1 and SEC4, which are found at the inner surface of the plasma membrane^{22,23}, but are not involved in chemical transduction. Rather, these proteins seem to function as mechanotransducers, in that mutations in these genes disrupt microtubule organiza-

tion (YPT1, ref. 22) or abolish late steps in normal secretion (SEC4, ref. 23). The *ras* family may therefore act as a relay between surface receptor activation and structural changes in the cytoskeleton. If so, this suggests that the efficacy of signalling pathways is altered by spatial reorganization of transduction elements. □

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Cytoskeleton

Protean proteins perceived

Walter Gratzer

DR Johnson's Dictionary of 1755 gives the following majestic definition of a network, and one that will serve for the purposes of cell biology: "any thing reticulated or decussated at equal distances with interstices between the intersections." Such, for example, is the red cell membrane cytoskeleton, which is a stable complex, designed to endure for the lifetime of the cell. The dynamic structures that are assembled from the basic cytoskeletal elements and dismantled to suit the requirements of the cell, are more variable and varied, and their discovery has brought in its train a whole gallery of new proteins with properties never envisaged in the days when all proteins were either fibrous or globular. Some of these molecules will undergo remarkable shape changes in response to minimal environmental perturbations. The study of these phenomena has led to a return to the quantitative virtues of physical protein chemistry and a resurgence of ancient crafts: the whine of the Model E* is once again to be heard in the land, like the voice of the turtle, to gladden the hearts of the old-timers.

One of the most interesting and instructive of recent studies along these lines comes from W.P. Lynch, V.M. Riseman and A. Bretscher (*J. biol. Chem.* **262**, 7429; 1987) and concerns caldesmon, a protein found in some abundance in smooth muscle and also in stress fibres in other cell types. There has been dispute about its physical properties and about what it does and how it does it, but it is certainly an actin- and calmodulin-binding protein, and there is a high likelihood that it exerts some form of calcium-dependent control over actomyosin activity. Caldesmon preparations from chicken gizzard contain two closely similar polypeptide

chains, running in SDS gels with apparent *M_s* of 135,000 and 140,000, and the molecule has been variously reported to be a monomer and a dimer, globular and elongated.

Lynch *et al.* have resolved the conflict and the answer is of an unexpected kind. The molecule in dilute solution is clearly a monomer, and it presents a highly elongated, though rather flexible, appearance in the electron microscope. In this it resembles many other cytoskeleton-associated proteins. When an oxidizing agent is introduced to convert thiols into disulphide bridges, caldesmon forms a progression of oligomers, together with a compact form of the monomer, which must have an intramolecular disulphide bond. This is probably a unique compact conformation, for the evidence is that the molecule possesses only two cysteines.

Such intramolecular crosslinking is not unknown—for example, the 100-nm-long spectrin dimer can be made to eat its tail by oxidation (Becker, P.S., Cohen, C.M. & Lux, S.E. *J. biol. Chem.* **261**, 4620; 1986). What is extraordinary is the stability of the oxidized form, which is refractory to all but millimolar concentrations of the powerful reductant dithiothreitol. It is true, of course, that real globular proteins containing disulphide bonds are generally resistant to reduction except when the interior of the molecule is exposed with the aid of denaturants. The internally oxidized form of caldesmon thus probably has a globular fold of considerable stability. It would certainly be expected to resist the much weaker reducing conditions (glutathione) prevailing in the cell. If the oxidized form indeed occurs *in vivo* this would constitute a most unusual situation, for disulphide bonds are normally found only in extracellular proteins (in practice those which come in

*Not an early car, but the Beckman analytical ultracentrifuge.

1-gram bottles, like albumin, pancreatic ribonuclease or lysozyme).

This is not the end of the story, however, because caldesmon has been found by some workers, but not by others, to bundle actin filaments. This activity, it now turns out, is not a property of the monomer, which is evidently univalent with respect to actin, but of the disulphide-linked oligomers. The actin bundles thus disappear on reduction. The location of the cysteines and the relation of the bulk of the molecule to the actin-binding region, which is quite small, should make an interesting sequel.

A different version of the behaviour of caldesmon has also now appeared, however. R.A. Cross *et al.* (*FEBS Lett.* **219**, 306; 1987) detected by gel filtration the salt-dependent formation of presumed end-to-end dimers. If reducing conditions were used, the separation on the column must imply that there is no rapid association-dissociation equilibrium. Why this should be, and how it relates to the observations of Lynch *et al.*, who evidently saw no such effect, is by no means apparent.

Chicken gizzard contains, among further good things, another actin-binding and crosslinking protein, talin. This molecule is found, together with vinculin, in focal adhesions and is conjectured to assist in the attachment of actin bundles to the membrane. Talin, too, has interesting and unusual physical properties. L. Molony *et al.* (*J. biol. Chem.* **262**, 7790; 1987) find that, at least at low concentration, the protein has a M_r (by sedimentation equilibrium) of 225,000, which is the same as the value delivered by SDS gel electrophoresis. It is thus a monomer, and again appears highly elongated and flexible in the electron microscope. Unlike caldesmon, but like spectrin for instance, the conformation is predominantly alpha-helical. The hydrodynamic properties (again like spectrin) are salt-dependent but, perhaps uniquely, the sedimentation coefficient falls, and thus the Stokes radius (which Molony *et al.* also measured by gel filtration) rises with addition of salt. The molecule evidently collapses at low ionic strength and elongates to something approaching a rod at high. Moreover, when the protein concentration is increased the chains associate but only, according to ultracentrifugal analysis, to a dimer. A reasonable picture of what goes on in the cell is that the talin exists as a monomer in the dilute pool present in the cytoplasm. This would be in equilibrium with the dimer in the highly concentrated protein complex that makes up the focal adhesions, where it can crosslink and collect up the actin, vinculin and the fibronectin receptors, to all of which it will bind. Protein chemistry lives yet. □

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Micrometeorites

Secrets of black dust revealed

Ian D.R. Mackinnon

YET another source of micrometeorites has now been found, this time on the Greenland ice cap, thanks to the detective work of Michelle Maurette and colleagues. This discovery, first announced last year¹, opens up a rich area of investigation for those keen to extract every shred of information from debris of the smaller bodies of the Solar System, such as comets and asteroids. The recent comet Halley fly-by (see *Nature* **321**, 259–366; 1986) and developments in the study of interplanetary dust particles (IDPs) collected from the stratosphere have whetted the appetite of cosmochemists, and the addition of a new, abundant source of unaltered micrometeorites of unprecedented grain sizes will further quicken the pace of progress. Maurette *et al.* have now followed their earlier announcement¹ on the discovery of Greenland cryoconite (or black dust) with a detailed analysis of the extraterrestrial component from this new source, reported on page 699 of this issue².

Chemical and petrographical observations on six basic types of cryoconite grains confirm their extraterrestrial origin. The data also suggest that the Greenland deposits contain micrometeorites that are unaffected by their entry into the atmosphere and are of a size range not previously observed in collections of IDPs. As well as identifying new types of extraterrestrial grains, Maurette *et al.* compared their size distribution and total flux during recent time with that for micrometeorites in orbit at 1 astronomical unit (AU)³. This comparison of extraterrestrial cryoconite and interplanetary dust fluxes suggests to Maurette *et al.* that the sources of these grains are not larger bodies, such as centimetre- or metre-sized meteoroids, but are indeed micrometeoroids of less than 1 mm in size.

Greenland is not the only place on Earth where space debris (both man-made and natural) can be collected. Micrometeorites (or cosmic dust) less than 1 mm in size have been successfully collected in deep-sea sediments of the Pacific Ocean, in the lower stratosphere and, with modest success, in ice cores from Antarctica. The Greenland deposits offer a much greater abundance of the larger fraction (>100 µm), and seem to be less affected by terrestrial weathering processes. The abundance of 100-µm micrometeorites in the Greenland cryoconite is 100–1,000 times greater than that determined for deep Pacific Ocean sediments because of a unique concentration mechanism¹, similar to the processes involved in the accumulation of larger

(greater than centimetre-sized) meteorites in Antarctica. A similar coordinated collection effort in Greenland may be as rewarding as the Japanese and American Antarctic meteorite expeditions. For the new micrometeorites, the concentration mechanism involves incorporation of extraterrestrial particles in the relatively unpolluted Greenland ice sheets, glacial transport to coastal regions and seasonal melting of these ice sheets. The liberated extraterrestrial particles are then carried by the seasonal runoff to form as placer deposits in shallow basins of the Greenland coastal regions.

The fundamental basis for these calculations on the micrometeorite flux over the past 3,000 years is a well-characterized glaciology, supported by ice-flow models for the Søndrestromfjord collection site. By taking into account variations in ice-flow rates over the collection area, the mass fraction of collected micrometeorites per mass of cryoconite and assuming a constant micrometeorite flux over the past 3,000 years, Maurette *et al.* estimate that each kilogram of collected cryoconite corresponds to an exposure of 0.5 m² of surface for about 3,000 years. The only comparable exposure surfaces for recent micrometeorites of this size range seem to be the large-area collection surfaces that will be used in the forthcoming stratospheric-collection flights sponsored by NASA. Even then, these surfaces would need to be flown for impractical periods of more than 400 hours to obtain a statistically viable dataset of 100-µm or larger micrometeorites.

Even with approximate models for the apparently stable environment at Søndrestromfjord, the magnitude and shape of the size distribution for more than 2,000 grains from the Greenland cryoconite compare remarkably well with that compiled by Grün *et al.*³ from more than 15 years of satellite observations on the micrometeoroid flux at 1 AU. Also, size distributions for two of the most abundant particles, the chondritic-stony and oxidized Fe/Ni spheres, are quite distinct from those known to occur for particles formed by repeated impacts on planetary surfaces. The analysis by Grün *et al.* establishes that within the mass range appropriate to Greenland cryoconite (>10⁻⁵ g), catastrophic collisions dominate particle lifetimes and that, subsequently, these particles are lost from 1-AU orbits. To maintain a steady-state distribution at 1 AU over known orbital lifetimes, particles in this mass range have to be replenished by interplanetary sources. The most

commonly accepted sources for micrometeoroids at 1 AU are short-period comets, which Maurette *et al.* believe produce the extraterrestrial component of Greenland cryoconite.

Grün *et al.*, however, suggest a significant contribution to the micrometeoroid complex at 1 AU is from collisions between asteroids crossing Earth's orbit and asteroid fragments. This notion arises from the suggestion that the present-day input from comets is insufficient to replenish the observed micrometeoroid flux⁵. The debate has been joined by workers studying the smaller-sized fraction (<50 µm) of chondritic IDPs collected in the stratosphere. The sources of these smaller particles are also of great concern and many have argued for an asteroidal component since the publication of the Infrared Astronomical Satellite (IRAS) results on extensive dust bands around the asteroid belt. The proportion of asteroidal or cometary debris that makes up the present micrometeoroid flux at 1 AU, and that entering the Earth's lithosphere, are critical parameters in this debate. Nevertheless, whatever the primary source of the extraterrestrial component in Greenland cryoconite, it is clear that these grains are freshly derived from their interplanetary orbits (<10⁴ years).

Of more immediate interest is the type and nature of micrometeorites discovered in these Greenland placer deposits, in which there are at least four types of extraterrestrial cryoconite grains either not observed or extremely rare in deep-sea collections. These include unmelted chondritic fragments, two types of glassy spherules, and unmelted, unoxidized Fe/Ni particles. Further, because the Greenland ice layers sample a shorter period than the 10,000-year span of deep-sea sediments, the degree of alteration of cryoconite micrometeorites is considerably reduced compared with deep-sea micrometeorites. The Greenland samples also benefit from less severe weathering processes on Earth¹, accounting for the preservation of new types of micrometeorites. Alteration does occur, however, and it is different in the same type of olivine-rich, stony spheres in deep-sea sediments and in the Greenland cryoconite.

A study by Callot *et al.*⁶ shows that weathering of stony spherules in the Greenland ice cap is related to the presence of siderobacteria, the millimetre-sized cocoons of microscopic filaments comprising much of the Greenland cryoconite. In deep-sea spherules and spherules accumulated in high-purity melt-water ponds in Greenland (devoid of biogenic agents), olivines etch at a faster rate than olivine in cryoconite spherules. Thus, not only do these Greenland deposits present new types of micrometeorites from a previously poorly sampled size range, they also seem

to preserve the original mineralogy and petrology of most extraterrestrial cryoconite grains.

It is the sampled size range of the micrometeorites that is perhaps the most exciting aspect of their discovery. Micrometeorites between 100 and 300 µm are much more amenable to isotope and trace-element analyses from individual grains or components of these grains than the smaller IDPs collected from the stratosphere. Indeed, preliminary trace-element data using synchrotron X-ray fluorescence have already been reported for individual iron and chondritic spherules from Greenland cryoconite deposits⁷. The presence of large (300 µm) unmelted chondritic grains with minimal terrestrial alteration could spark another series of searches for anomalous isotope behaviour in refractory components if they occur in these Greenland particles. According to Maurette *et al.*, the nature of these unmelted chondritic grains, with some suggestion of porosity, indicate an efficient cooling mechanism for incoming micrometeoroids. This suggestion contradicts the conventional view on the ability of compact micrometeoroids to survive entry into the Earth's atmosphere⁸, but offers the hope that significant quantities of large micrometeoroids with the right combination of density, melting point, entry velocity and angle of incidence can be collected without alteration by atmospheric ablation or weathering. Indeed, in one fell swoop, Maurette *et al.* increase the potential database on this unique subset of the micrometeorite flux by more than 250 times (see Table 2 on page 701 of this issue).

Maurette *et al.* cite the survival of the larger micrometeorites and their inferred low density as additional support for a cometary origin of these particles. In particular, they suggest that the unmelted chondritic fragments larger than 100 µm may be derived from the non-volatile fraction of dark cometary nuclei. This thesis is difficult to test, as the only comparable data currently available, from comet Halley and the stratosphere, come from grains that are considerably smaller in size (or mass). There is some support for the presence of larger, even boulder-sized, grains on comet nuclei based on more recent models of comet morphology⁹, and it is reasonable to suppose that on jetting, grains with masses >10⁻⁵ g are released during passage through the inner Solar System. A more plausible, though less-frequent, mechanism for the intersection of larger-sized cometary particles with Earth's orbit³ could occur through the break-up of comets in the inner Solar System. □

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Ocean Drilling Program

Palaeoceanography of the subantarctic South Atlantic

*Leg 114 shipboard scientific party**

DESPITE experiencing gale force and stronger winds during 29 days of the cruise and combined seas of 4–5 metres on a routine basis, the vessel *JOIDES Resolution* drilled twelve holes at seven sites along an east–west transect of the subantarctic South Atlantic during Ocean Drilling Program Leg 114 (Fig. 1). The recovered sediments (cored in water depths ranging from 1,807 to 4,634 m) provide an almost complete composite section spanning 90 Myr, from the Late Cretaceous to the Quaternary. Two-thirds of the recovered sediments are of Late Cretaceous–Paleogene (90–23.7 million years, Myr) age and are primarily pelagic carbonates which may provide a valuable oxygen and carbon stable isotope record of the subantarctic region. These sites and those recently drilled by ODP

Leg 113 in the Weddell Sea (see the recent News and Views article in *Nature* **328**, 115–116; 1987) provide the first detailed sedimentary record of the paleoenvironmental evolution of a single sector of the southern Ocean.

The geochronology obtained by Leg 114 is crucial for the interpretation of the influence of the Antarctic ice sheet on the world's oceans and climate in the Cenozoic. The palaeoceanographic history of the South Atlantic was influenced by tectonic events, such as the opening of the Tasmanian Seaway and the Drake Passage between South America and Antarctica. These and other events led to drastic changes in latitudinal and vertical oceanic thermal gradients and ocean-circulation patterns. Until now there has been no detailed chronology of

the evolution of the southern oceans, preventing recognition of the dynamic relationship of the Antarctic region with the global oceanographic-climate system.

The stratigraphy established by Leg 114 will make it possible to evaluate changes in the subantarctic from the late Cretaceous (90 Myr ago) to the present, during which the climate has changed from subtropical to temperate to sub-polar. Also we shall evaluate the influence of regional tectonic activity on the glacial and oceanic history of the southern Ocean.

We obtained well-preserved assemblages of all important microfossil groups. Thick, relatively complete Palaeocene sequences (65–24 Myr) provide detailed biostratigraphy. Palaeomagnetic data provide a near-complete history of geomagnetic polarity reversals since the late Cretaceous (90 Myr ago), with gaps only in the late Palaeocene to early Eocene and the early to middle Miocene. Previous data were limited to the late Neogene (the past 8 Myr). The new information will provide the first nearly complete magnetostratigraphic timeframe for calibration of Cenozoic, high southern latitude biostratigraphic zones.

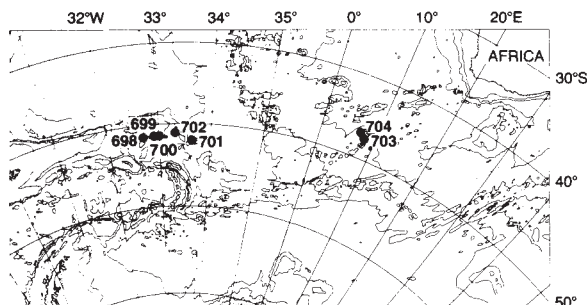


Fig. 1 Twelve holes were drilled by ODP Leg 114 at the seven site locations shown along a west to east traverse across the subantarctic South Atlantic (map with 3,000-m isobath).

Our new data constrain models for the complicated tectonic evolution of this region. The north-east Georgia Rise is at least as old as late Cretaceous (90 Myr ago), was once subaerially exposed, and may have a continental root. The Islas Orcadas Rise was formed during the late Cretaceous to the early Palaeocene (90–62 Myr ago), as was Meteor Rise although its volcanic history continues into the Eocene (50 Myr ago). These features, regional fracture zones and plateaux, obstructed communication between the deep and intermediate waters of the Antarctic and South Atlantic. The separation of the Islas Orcadas and Meteor Rises by Eocene seafloor spreading created a deep-water gateway, allowing circulation during the mid-to-late Palaeogene (50–24 Myr) that was more vigorous than contemporaneous circulation at shallower sites.

Cretaceous (90 Myr ago) to middle Eocene sediments contain a mixture of austral and warm-water microfossils, suggesting a mingling of Pacific and low-

latitude Atlantic surface waters. Although Drake Passage was closed at this time, austral assemblages may have migrated into the South Atlantic through a shallow unglaciated West Antarctic seaway between the Pacific and South Atlantic. Circulating, subtropical South Atlantic water probably mixed with the Pacific waters, bringing low-to-middle-latitude assemblages to the subantarctic. Meridional thermal gradients were weak and deep waters were suboxic because of weak vertical mixing.

Microfossils with warm-surface-water affinities were abundant during the early Eocene, probably the warmest period of the Cenozoic. Surface waters began cooling significantly by the earliest middle Eocene (50 Myr ago) and by the late Eocene (38 Myr ago) most warm water species had disappeared. Further cooling occurred during the Oligocene between 33 and 27 Myr ago and late Oligocene assemblages are dominated by cosmopolitan and cool-temperature species.

During the earliest Miocene (23 Myr ago) the Antarctic Convergence moved to 51.5° S, signalling the expansion of the southern ocean Antarctic surface waters and the establishment of the Antarctic Circumpolar Current. After the later early Miocene, assemblages show pronounced provincialism because of increased thermal isolation from the lower latitudes. Only in the eastern subantarctic (site 704) was there any significant temperate-to-subtropical influence during the Neogene.

All five sites west of the Mid-Atlantic Ridge were found to have large hiatuses between upper Palaeogene to lowermost Neogene and younger Miocene sediments. Physical properties across most of these hiatuses indicate large-scale removal of sediment. We suggest that these hiatuses were caused by the formation of a deep-reaching circumpolar current, in turn caused by the opening of Drake Passage. Hiatuses representing a greater interval in the shallower sites imply that either the currents there were faster or that erosion persisted longer there when the passage first opened.

Site 704, the most northerly site (46° 53' S), remained north of the Antarctic surface waters and the circumpolar current throughout most of the Neogene without long periods of nondeposition or erosion. A lower Oligocene–Quaternary sequence was recovered at this site that contains the thickest (576 m) and most complete Neogene sequence yet obtained from the subantarctic. The geochemical log of the hole and detailed carbonate analyses (Fig. 2) confirm the presence of strong climatic

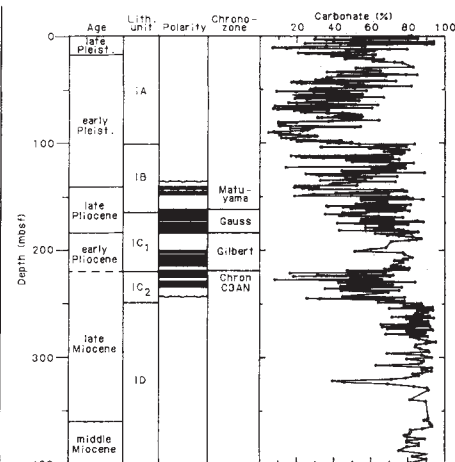


Fig. 2 Fluctuations in the percentage of calcium carbonate in the lower Oligocene–Quaternary section of site 704 on the Meteor Rise. The advent of high-frequency, large-amplitude fluctuations in carbonate content represents the beginning of strong glacial–interglacial variations.

cycles on the scale of tens to hundreds of thousands of years. Future studies of the isotope distribution, palaeontology, sedimentology, and other characteristics of this site should provide an unequalled southern, high-latitude reference section of Neogene climatic history.

Preliminary analyses of site 704 data indicate that the late Oligocene to middle Miocene period (30–13 Myr) was environmentally more stable than the period since. Little change occurred in sedimentation during the middle Miocene; however, cooler assemblages of planktonic organisms appeared by 13 Myr ago. A pronounced cooling trend began during the late Miocene (9 Myr ago), after which the sediment record is strongly cyclical, revealed by carbonate fluctuations (Fig. 2) and in the geochemical logs. There was severe cooling during the latest Miocene (6.5 Myr ago) to earliest Pliocene (5.0 Myr ago) followed by the last brief period of climatic stability.

During the late Pliocene (2.9 Myr ago), the Antarctic convergence moved closer to the site, leading to an increase in the deposition of biogenic silica and the advent of strong cyclic sedimentation. Late Pliocene–Quaternary cyclic deposition was controlled primarily by glacial–interglacial fluctuations of calcareous and biosiliceous sedimentation and carbonate dissolution. □

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On the sombre view of AIDS

SIR—“The sombre view of AIDS” by Malcolm Rees (*Nature* 326, 343; 1987) presents a more pessimistic picture than hitherto described, based on a data analysis in which the incubation period for AIDS (acquired immune deficiency syndrome) is estimated to have a mean of 15 years and standard deviation 5 years. He does not state how the estimation is performed. Barton (*Nature* 326, 734; 1987) pointed out that the data analysis seemed to be incorrect and that the mean is probably much more like 5 years. His analysis is based on a more nonparametric approach (Rees assumed that AIDS incubation time is normally distributed) and takes some account, but not full account, of the fact that the data are truncated (see below). The 5-year estimate is itself somewhat *ad hoc*; it is not given by a formula. I also believe the data analysis of Rees to be incorrect, and I should like to explain how a proper analysis based on the normality assumption, but taking full account of truncation, can be carried out. Such an analysis entails a formula for the mean and standard deviation. It confirms Barton's observation that the data support a mean value of 5 years, a number which is similar to other estimates of mean incubation time (Peterman, T.A. *et al.*, *J. Am. med. Ass.*, 254, 2913–2917; 1985), and, therefore, also supports a considerably less pessimistic picture.

The data consist in part of the time from infectious blood transfusion to AIDS diagnosis for each of the 144 patients with blood-transfused AIDS reported to the Centers for Disease Control (CDC) during the period 1978–84(5). From these data, the mean and standard deviation of the probability distribution of AIDS incubation times are to be estimated. The author suggests that the estimation be performed under the assumption that this distribution is normal.

Each one of the 144 people reported to the CDC and transfused in 1978 can have an incubation period of no more than 7 years, which I call the truncation time associated with 1978. Similarly, each of the 144 persons transfused in 1979 can have an incubation period of no more than 6 years, the truncation time associated with 1979. In general, with each of the years a person in the sample could have become infected, there is an associated truncation time. Under the normality assumption, the probability distribution of the incubation time of a sampled individual is a truncated normal distribution (*Continuous Univariate Distributions – I*, Johnson, N.L. & Kotz, S., Houghton Mifflin Co., 1970) with truncation time determined by the year the individual was transfused and which is described above. Consequently, among

the sampled individuals, the distribution of the incubation time is individual-specific (the truncation times vary between individuals, but are known constants). But these distributions share the two parameters of interest, the mean and standard deviation of the underlying normal distribution for the general population of infected individuals. One may fit these distributions to the data the author presents in his Table 1 using the classical statistical procedure of maximum likelihood (*Theoretical Statistics*, Cox, D.R. & Hinkley, D.V.; Chapman and Hall, 1974). The incubation times are reported in years, rather than on a “continuous” scale, giving rise to many tied observations. However, the truncated normal continuous scale can be appropriately discretized by taking the probability that the reported incubation time for a sampled individual is i years to be the difference in the values of the individual's (cumulative) truncated normal distribution function at years i and $i-1$. Then the product of these probabilities, taken over all sampled individuals, is maximized with respect to the two underlying parameters, mean and standard deviation. The values of these parameters giving the maximum are the desired estimates. Using this approach, the mean and standard deviation parameters are estimated to be 4.51 and 1.56 years, respectively. The standard error estimates for these parameters are 0.468 and 0.176 respectively (as obtained from the inverse estimated information matrix).

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Defending ‘pragmatypes’

SIR—Endrödy-Younga (*Nature* 327, 664; 1987) rejects my proposal for ‘pragmatypes’ (*Nature* 326, 251; 1987) first, with reference to Recommendation 75E of *The International Code of Zoological Nomenclature* (1985 edition). This is unacceptable not only because a Neotype, by definition¹, replaces a lost type (as opposed to an existing type) but because Recommendation 75E is itself unacceptable. It is so worded that, taken at its face value, it succeeds in being an impediment to science. This is discussed elsewhere².

Second, Endrödy-Younga is alarmed by the prospect that a pragmatype may, with advance in knowledge, itself become an impediment and have to be replaced by a subsequently designated new pragmatype. Why is this a problem? Types are designated in order to assist the resolution of problems of nomenclature that may arise with advance in taxonomic understanding.

They are not, despite the attitudes of certain museum curators, analogues of antiquities such as the Elgin Marbles. It is the advancement of science that matters not the preservation of the status of particular biological specimens.

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Maturational patterns in early hominids

SIR—In a letter to *Nature*, Smith¹ finds that certain early hominid jaws and associated teeth show an ‘ape-like’ pattern in the timing of dental calcification. She concludes that these early hominids had foreshortened growth and development periods similar to modern apes. We find a number of problems with Smith's interpretations of these dental data.

First, numerous studies of human dental calcification and eruption document that no single sample's mean ages for these features adequately represent diverse values for individuals in human populations (see, for example, refs 2–7). Individual tooth calcification stages may vary by as much as 3–5 years within and between samples. Therefore, the ages assigned by Smith to the fossil teeth do not represent their actual ‘dental ages’ but are sample-dependent mean values⁵. The problem is that while Smith claims to demonstrate evolutionary patterns in dental development by two approaches, ‘fit to standards’ of apes and humans and patterns in the plots of individual teeth relative to that of the M1, we find that a similar analysis of dental calcification stages in ten modern human specimens and of the fossil specimens presented by Smith shows variable patterns depending on the human standard-mean ages used^{2–6}. Modern humans are ‘best fit’ to pongid standards and characterized by a ‘primitive pattern’ in eight out of ten cases¹. Thus the method is clearly invalid. There is no *a priori* method which directs the choice of one human sample's means over another to analyse these fossils, as none can be said to be more closely related to the fossil sample under study than any other.

Second, Smith misuses growth standards, confusing data on a group with that for an individual. The age of a child is not often equal to a mean age derived from individual teeth. Synchronous teeth from individual children are not of equal age as assessed from sample means (as assumed by Smith) but can differ by as much as 2.25 years⁸ (see Table 1). Furthermore, pub-

Table 1 Comparison of fossil specimen dentition with that of modern human children

a	8 Years of age	ER-1590	8-Year-old human male ⁸
Tooth	calcification stages ²	Stage Mean age ¹	Stage Mean age ¹
I1	R _{3/4} — A _{c-}	R _{3/4} 6.0	R _{3/4} 6.0
I2	R _{1/2} — A _{1/2}		
C	R _{2/3} — R _{2/3}	Cr _c 4.0	R _{1/4} 5.75
P3	R _{1/6} — R _{1/2}	R _{1/3} 7.1	R _{1/4} 6.9
P4	Cr _c — R _{1/4}	R _{1/3} 8.2	R _{1/4} 8.0
M1	R _{3/4} — A _{1/3}	R _c 7.3	R _c 7.3
M2	Cr _c — R _{1/4}	Cr _c 6.4	Cr _c 6.4
M3	Crypt — Cr _i		
b	Age (years)	Specimens	
	4 – 5	OH 30, KNM – ER 1477, LH2	
	5 – 6	Gibraltar, LH3, Sts 24, SK 852, KNM – ER 1820	
	6 – 7	SK 63, KNM – ER 1507, ER 1171, ER 820	
	7 – 8+	Sts 151, KNM – ER 1590	

a, The range of calcification stages² and mean ages of attainment¹ for a single 8-year-old human male child⁸. The dispersion of dental ages of the teeth around the M1 in this modern human child is described by Smith's primitive pattern. Note the range in mean ages of attainment for each individual tooth. The stages of dental development in KNM – ER 1590 fit well within this age range. b, The hominid specimens presented by Smith giving the age of the appropriate human dental complexes.

lished reference standards present standard deviations about the mean age at which dental developmental stages are achieved in a group of children, not standard deviations between ages of teeth in a single individual, as Smith assumes. These misconceptions lead her to conclude, incorrectly, that some fossil hominid specimens cannot be human-like because 'differences of 4 to 6 s.d. units exist between synchronous dental events in an individual'. This would also be true for many modern human children^{2,8}. When the fossil specimens presented by Smith are compared to complete dentitions of modern children, nearly precise overlap occurs for all specimens (Table 1).

Finally, Smith's choice of young specimens, along with her failure to consider calcification of the M3, make it impossible to use the very significant absence of the M3 in her analysis. Considering the range of variability in human dental development and the paucity of comparable information on pongids⁹, it may be possible to fit young specimens into either a human or pongid age-specific array of calcification stages. Thus, delay of M3 calcification in humans appears to be the most reliable of the features distinguishing apes

and humans and one the early hominids do share with humans^{10,11}.

In sum, although no definitive evidence exists for the rate of dental development in early hominids, the current data reveal a clearly human-like pattern of dental calcification.

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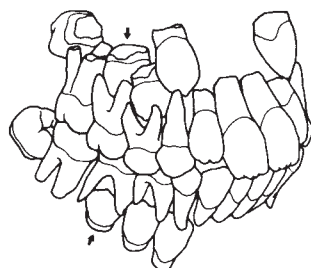
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SMITH REPLIES — Mann *et al.*¹ defend previous views² that early human ancestors share a human pattern of growth and development, objecting to my recent conclusion³ that most early hominid specimens resemble apes more closely than modern humans in development. They argue that early hominid fossils can be accommodated in a human pattern if (1) other standards of human dental develop-



Immature dentition of the child of about eight years from the atlas of van der Linden and Duterloo¹⁵ (skull 14, p. 126). Note small amount of root formation of unerupted last permanent premolars (arrows) *contra* Mann *et al.*¹ Table 1 (drawn by Shayne Davidson).

ment are substituted for those I used, and if (2) large deviations are common in dental ages within individuals.

Mann *et al.* suggest use of growth studies⁴⁻⁶ I specifically reject. The history of Schour and Massler⁴ is now well known^{6,7}. These authors reprinted values of Logan and Kronfeld^{8,9}, surgeons who were primarily interested in avoiding damage to unerupted teeth during surgical repair of cleft palate. Their chronology of dental development was based on dissection of 25 children, 19 of whom were under two years of age. Five additional children of undescribed age were added by 1935 (ref. 9), but probably only six children provided information for ages two to 15 years. Further, the children were debilitated and some were developmentally abnormal⁸.

Nolla⁵ deciphered tooth formation stages from ~3,400 radiographs of 50 children. But Nolla was apparently unaware of the inevitable truncation of age distribution of subjects and the positive skew of all growth data that make standard mean and variance calculations inappropriate. Data on age of attainment of growth stages are properly analysed with cumulative distribution functions¹⁰⁻¹², usually supplemented with probit or related analyses^{11,12}. Nolla's analysis has further difficulties: ranked data were treated as continuous, and curves describing mean stage for age were apparently solved secondarily for age, a procedure analogous to solving regression equations for the independent variable.

Given the importance of statistical analysis, Fass⁶ must be rejected for sparse information: '... the recorded information was totalled on each page for each stage of growth and development' (p. 394). Fass also does not mention the number of children in the study, only the number of radiographs.

The remaining set of radiographic studies is based on large numbers of clinically-normal children from Ohio⁷, Boston¹³, or Ohio plus Boston¹⁴. All describe appropriate statistical procedures. Of these, the last and most complete is the Moorrees *et al.*¹⁴ study of 345 children, the standards I used³.

Large variations in reported mean ages of attainment of tooth formation stages appear to reflect methodological problems rather than human variation. The great range of stages in Table 1 of Mann *et al.*¹ cannot be accepted as these are based on Nolla⁵.

Mann *et al.* also argue that widely-varying dental ages of ER 1590 *Homo habilis* are within human limits similar to those of a particular 8-year-old human child in ref. 15 (see figure). Stages of root formation given for this child in Table 1 of Mann *et al.*¹ differ somewhat from my assessments for most teeth, but the assignment of 'root 3/4 complete' to the last premolar, the source of the claimed devia-

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- Conroy, G., presented at *Am. Anthropol. Ass. meetings*, December 1986.

tion, is grossly incorrect. The highly deviant pattern attributed to this child results from inaccurate formation stages combined with inaccurate standards. In addition, the true age of the child is unknown; van der Linden (personal communication) assigned the age of eight years based solely on dental age.

The impression given by Mann *et al.* that five- to six-year deviations in dental ages of teeth within individuals are common is contrary to published dental ages¹⁶, the known high intercorrelation of dental development¹⁷ and common experience. I do not deny the existence of variation (nor do I hold assumptions about variation ascribed to me¹), but the argument that humans and apes are highly variable does nothing to further the case that early hominids can be regarded as human-like in growth. To the contrary, most of the early fossil hominids are both poor humans³ and good apes¹⁸ in developmental pattern, and a whole series of fossils, spanning millions of years, share a fairly typical ape-like pattern of development.

Fossils showing third molar calcification would be valuable additions, and future radiographic or scanning studies may provide these data. But one of the most interesting findings of new work on great apes¹⁹ is that molar formation is not nearly as accelerated in these forms as had been supposed². Findings for third molars of the Swartkrans hominids (the absence of M3 during eruption of M1 [SK 63²] or the complete crown of M3 combined with completed M1 and advanced M2 root [SK 843²]) would be expected in humans¹⁴, great apes¹⁹ and macaques²⁰. As this suggests, not all stages are diagnostic, and

some portion of molar delay in higher primates probably represents a growth differential that separates anthropoids from prosimians.

Mann is a pioneer in the design of a question and a method, and especially in realizing that information as dynamic as pattern of growth and development might be retrieved from the hominid fossil record. Yet, new data, methods, and approaches^{3,14,19-22} promise to enlarge our knowledge of the evolution of human growth and development.

B. HOLLY SMITH

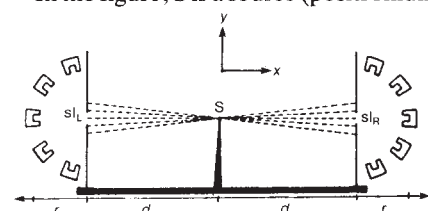
*Museum of Anthropology,
University of Michigan,
Ann Arbor, Michigan 48109, USA*

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Popper versus Copenhagen

SIR—I am grateful to Collett and Loudon¹ for opening up a discussion of my 1982 proposal² for a crucial experiment between the (subjective) Copenhagen Interpretation of quantum mechanics and my own (objective) propensity interpretation—an experiment based upon Einstein, Podolsky and Rosen³ and a radical simplification of another proposal by myself⁴. With the aid of a figure, let me briefly restate the experiment before replying to Collett and Loudon's criticism.

In the figure, S is a source (positronium,



say). Some of the pairs of particles that are emitted together, but in opposite directions, pass through the slits sl_R and sl_L of screens at equal distances (d), right (R) and left (L) of the source. After that they

scatter and trigger coincidence detectors (at distance r). The width of sl_R and sl_L can be varied and, if the slits are narrowed, the range of the scatter becomes wider (diffraction) and vice versa. Now we remove the left screen. What will happen to the left range of scatter?

My prediction is that the diffraction scatter on the left disappears and the remaining scatter, by contrast, increases with increasing width of sl_R . The prediction of the Copenhagen interpretation is that the diffraction scatter on the left does not disappear and, as before, it becomes wider if we narrow sl_R because we indirectly measure the y -position of the particle on the left when it reaches $x = -d$, and it is our measurement or, according to Heisenberg, our knowledge, that creates scatter by the uncertainty principle. So the two interpretations arrive at opposite predictions; that is to say, we have a crucial experiment.

Collett and Loudon, although not very explicitly, agree with all this provided the source is fixed. But they say that the source must not be regarded as fixed: it is subject to (Heisenberg's) uncertainty

principle. By an intricate analysis (open to severest criticism) they arrive, if I understand them correctly, at the conclusion that my prediction is tenable but that the prediction of the Copenhagen interpretation leads to a clash with it. So I must not claim that the experiment is crucial; or as they put it: "In summary, it has been shown that source uncertainty effects in the experiment proposed by Popper remove the distinctive increase in left-hand beam divergence with reduction in right-hand slit width that he ascribed to the Copenhagen interpretation of quantum mechanics".

My answer is that the source is fixed. For we have, for its position and momentum:

$$\Delta y \Delta p_y = \Delta y \Delta v_y m \approx h \quad (1)$$

$$\text{and so } \Delta y \Delta v_y \approx h/m \quad (2)$$

But h/m can be made as small as we like. (Fixing our apparatus on rock, m becomes equal to the mass of the Earth.)

As to the "geometrical" uncertainty, discussed by Collett and Loudon, it does not matter that the source may reach from (say) y_1 to y_2 , as long as this width is reasonably small; for Δv_y of the particles may still be made as small as we like by making d sufficiently long (keeping r constant).

Incidentally, Collett and Loudon's title¹ is slightly misleading. My experiment was never intended as a crucial experiment of quantum mechanics but only of its (subjectivist) Copenhagen interpretation (which they call "the standard interpretation"). In fact there exist several interpretations of the formalism (as became clear in the recent Schrödinger centenary celebrations, for example, in Jon Dorling's contribution⁵). Also, formulae (3) and (4) of Collett and Loudon cannot be obtained from (1) and (2) the way they say, because the square of (2) which they use is an area measure. But something on these lines might be done if (2) were replaced by $\lambda/4\pi$. The results are incompatible with their findings.

KARL POPPER

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COLLETT AND LOUDON REPLY — We do not agree that the additional arguments made by Popper establish the ability of his proposed experiment to distinguish between the two interpretations of quantum mechanics he mentions.

One of the attractive features of the experiment as originally proposed by Popper was that the use of positronium as a source of particle pairs guaranteed that the two particles would come off in exactly

opposite directions (in the centre of mass frame of the source). Using a more massive source, not totally annihilated by the pair production, would indeed allow better localization of the source itself, but at the cost of introducing the complication of source recoil. The more massive the source, the less the effective constraint on the initial combined momentum of the particle pair (with respect to the centre of mass of the source). In the limit of a very massive source (say, the Earth), the directions in which the two particles were emitted would effectively be uncorrelated, and no conclusions at all could be drawn about the position of one from the detection of the other.

The net effect of the recoil for a massive source is that it really is Δp , of the source (as we used), not Δv , which is relevant in the end. One can get the same result without giving any consideration to the source at all, by realizing that the centre-of-mass position and momentum of the particle pair (in the laboratory frame) must themselves obey an uncertainty relation immediately after emission.

The geometrical contribution to the uncertainty may indeed be made small by taking Δy of the source to be small and d sufficiently long: this is implicit in our equation (5). In this case Δv , for the right-hand particle will also be small, as Popper says, but what is important is Δv , for the left-hand particle. This includes a contribution from Δp , of the source, which does not depend on d , and it is large if Δy of the source is small.

As regards the choice of scaling, our equation (2) is a unit of length: naturally its square is a measure of area. With this rescaling of the variables, equation (3) does follow directly from equation (1).

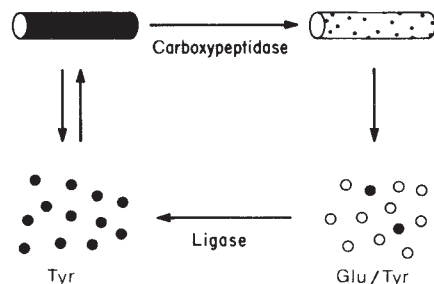
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A function for tubulin tyrosination?

SIR—In a recent News and Views article¹, Roy Burns reviewed some of the literature on tubulin tyrosination and proposed some models for the function of this unique post-translational modification *in vivo*. Unfortunately, Burns omitted to discuss some recent work that is relevant to the ideas he proposed.

The main tenet of Burns' article is whether the tyrosination state of tubulin protomers *in vivo* can regulate their ability to assemble into microtubules. Burns suggested that the detyrosinated (Glu) tubulin protomers in cells are compromised in their ability to polymerize into microtubules, whereas the tyrosinated (Tyr) subunits are polymerization-



Generation of distinct populations of microtubules by the cyclic tyrosination-detyrosination of α -tubulin.

competent. But in a recent study³, the polymerization-competence of Glu protomers *in vivo* was demonstrated directly by microinjection of Glu tubulin into cultured cells. Yet it is clear that, aside from this microinjection experiment, Glu protomers do not polymerize to form microtubules *in vivo*^{3,4}. This is not because of 'failure' of the Glu protomers to polymerize, but rather to the virtual absence of Glu protomers *in vivo* (we have determined⁴ that <2 per cent of the protomeric pool of tubulin is detyrosinated). This probably results from the rapid retyrosination of Glu tubulin in the monomer pool, as microinjected Glu protomer or Glu protomer resulting from the depolymerization of existing Glu microtubules is rapidly retyrosinated^{2,4}.

How, then, do microtubules with different levels of Glu tubulin arise in a common cytoplasm? Our experiments, originally reported two years ago³ and elaborated on in a detailed report⁴, demonstrate that microtubules distinct in their content of Glu tubulin are generated from a cycle (see figure) that involves polymerization of Tyr protomers, post-polymerization detyrosination of Tyr microtubules, breakdown of the resulting Glu-enriched microtubules and efficient retyrosination of Glu protomers.

In his article, Burns predicted that cellular microtubules would contain "a steadily increasing proportion of Glu α/β dimers with time, and hence with distance along the microtubule". It is obvious from the cycle we have proposed that growing microtubules would exhibit a gradient of Glu/Tyr ratios along their lengths. The slope and relative position of this gradient along the microtubule would depend upon the rate of polymerization of Tyr protomers and the rate of detyrosination by tubulin carboxypeptidase. However, we would not expect such a gradient to occur on microtubules that have amassed high levels of Glu tubulin, as these microtubules are not growing *in vivo*⁵ and, in fact, persist without addition of Tyr protomers for more than 16 hours⁶. This extraordinary longevity of Glu-enriched microtubules contrasts with that of most cellular microtubules, which persist less than 10 minutes. Whether Glu tubulin level is a cause or merely an effect

of microtubule stability, as Burns points out, is unknown.

In summary, existing data answer the question Burns posed concerning the mechanism of tyrosination/detyrosination. Further work should elucidate these unusual post-translational modifications: the most likely possibilities include the idea that Glu tubulin, which predominates in 'old' microtubules, is recognized by one or more specific microtubule-associated proteins to allow specialized function or interaction of that microtubule.

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RNA world

SIR—Prompted by the following quotes, I have written a short verse that may appeal to *Nature* readers.

"It was long thought that every cellular reaction is catalysed by a protein enzyme. The discovery that RNA can cut, splice and assemble itself overturns the principle — and throws light on early evolution."

T. Cech
Scientific American **255** (5), 64 (1986).

"[Cech's] discovery that RNA molecules can . . . act as enzymes makes the notion that life began from RNA very attractive."

E. G. Nisbet
Nature **322**, 206 (1986).

Primaevial proteins, earliest of enzymes,
Step aside. On the hydrothermal plane
It's an RNA world.
Slithering through the Archaean soup,
Improbable chains dabbed on ancient clays
Stain the basalt fabric,
And grimly splice themselves to bits.
Naturally selected, each is a list,
A preordained script;
Crudely but with dignity
Replicating catalytically. Stranded,
The detached daughters cling to rock.
Against the open ocean current, a coat of
lipids,
Thank you, would be welcome shelter.

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Let's hear it for science

Howard Simons

Selling Science: How the Press Covers Science and Technology. By Dorothy Nelkin. W.H. Freeman: 1987. Pp.224. \$16.95, £16.95.

THE mistake most people make when trying to evaluate the American press is to compare what it does with what it should do. What it does is anything but comprehensive, sometimes inaccurate and, at best, "catching history on the run", as it once was characterized. Most importantly the American newspaper is reactive, not active. Like the State Department, it has a crisis mentality and a crisis response. It has been late and inadequate on almost every trend in recent American society: consumerism, feminism, environmentalism, the energy crisis, civil rights, poverty. This leaves lots of room for criticism. And lots of room for academics, with measuring sticks and time, to try to work out how the American press does what it does and why it does it so poorly.

Take science reporting. It is roughly a half century since the first serious science reporting took root in the American newspaper. Most of the early journalism — and through a decade or so after the Second World War, when science became, as Paul Sears tagged it "the new superstition" — was primarily "oooh" and "aaaah" reporting of discovery, promise and progress. Reporters were, indeed, sorcerers' apprentices. When the country turned inwards in the 1960s, so did the science writers, who then concentrated more on such previously ignored areas as health care and ecological issues. Today, science and technology are such diffuse subjects that it is difficult to know just who is a science reporter. Science turns up in stories about military and foreign affairs, transportation, religion, economics, sport and so on.

Over this period, as science itself and the interest in it grew, so did the science writing cadre on newspapers large and small. Science writing now is a recognized speciality within journalism, with its own courses at colleges, organized seminars, professional organizations, fellowships, prizes, newspaper sections and professors who study it.

Now comes Dorothy Nelkin, a professor at Cornell University, to explore what it all has meant, means and should mean. Her thesis is not as much about "selling science" as it is about how American

science writers, over the past 50 years, have sold out to science. Her contention is that by holding science and scientists in awe, pandering to fashionable values, being passively uncritical and neglecting process in favour of promise, science reporters have seldom tackled "as news" the political questions of scientific responsibility and accountability or "the ideologies or social priorities that guide



science policy decisions".

Moreover, throughout her book Professor Nelkin hints darkly that science writers have been taken in by shrewd public relations, fashioned to sell science, by government agencies, universities, professional societies, industry, health groups and, heavens forfend, scientists themselves. She only nods in the direction of the so-called "good guys" who, with a lot less resources, have done remarkably well manipulating the American press in favour of one scientific or anti-scientific cause or another.

Reporting science and technology does tend to cheer-leading. Daily science reporting is susceptible to the press release or official source for its origin. Science writers do tend to depend almost wholly on what the reporting scientists say and often do not carry contrary voices, though in fairness it is often hard to find credible scientists willing to take on their colleagues and, if they do, for quotation. There seems to be a conspiracy of silence

among scientists when it comes to public criticism of one another's work. Would it help if more scientists wrote about science or more journalists trained in science? No one knows for certain. Professor Nelkin gives a good account of the plusses and minuses on both sides of this issue. But be mindful, as one newspaper wag said, "You don't have to be dead to write obituaries".

Nelkin has chosen a fascinating — one might even say important — subject that is rarely explored. But what she has done with it is annoying. The reason is that it is not at all clear how she has arrived at most of her conclusions — there is an infuriating lack of evidence for sweeping generalizations up with which no editor should put except in a book review.

Take this passage for example: "Often the style and content of reporting may have less to do with what is actually going on than with the promotional efforts of advocates, especially if these efforts coincide with journalists' perceptions of public attitudes and social biases" (pp.40–41). I would have relished an examination of how a reporter's or editor's lifetime inculcations affect what he or she leaves in a story or edits out. Such an analysis is not to be found here.

Although I expect selective blind quotes from journalists to underscore points, I hardly expect them from a scholar. Yet, this book is full of them, so much so that it is impossible to evaluate their worth.

And we get sentences like this one: "Humana Hospital gained

remarkable media exposure from the artificial heart, publicity that subsequently served its reputation and helped to fill beds..." (p.140). Perhaps. But she offers no evidence for the assertion that the publicity "helped to fill beds...". Or this generalization with only the dimmest substantiating data: "Except for the brief period in the late 1960s, press coverage of science has been shaped by the widely held belief that science is distinct from politics and beyond the clash of conflicting social values" (p.71). Oh to have been one of the many reporters who wrote cogently on the economic, social and political aspects of science and technology before and after the late 1960s and to have to read this.

Finally, how about this: "Newspapers must maintain circulation, attract advertisers, and not offend their advertisers, their owners, or their boards" (p.120). True, they must maintain circulation and attract advertisers, but where in God's name did the author dig up that business

about not offending advertisers, owners or boards? It sounds about as sophisticated as an anti-newspaper public relations person would have her believe.

What we have then is a conspiratorial view of the American science press; a view that suggests that American science journalists — undifferentiated by Professor Nelkin — are captives of the scientific establishment either because they are in awe of scientists or are intimidated by them. A view that suggests further that the scientific establishment, thereby, escapes the critical scrutiny that is afforded almost every other institution in the United States. A view that holds the American press buys what science sells and asks no questions.

"Scientists", Professor Nelkin con-

cludes, "must restrain the promotional tendencies that lead to controls on information or to oversell, and they must open their doors to more probing investigation. And journalists on their part must try to convey understanding as well as information. It is not enough to merely react to scientific events, translating and elucidating them for popular consumption..." (p.182). How else, she asks in this book, can the unwashed public understand the social, economic and political impact of science and technology on their lives and livelihoods? □

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Simian affairs

John C. Mitani

Apes of the World: Their Social Behavior, Communication, Mentality and Ecology. By Russell H. Tuttle. *Noyes Publications, Park Ridge, New Jersey 07656: 1987. Pp.421. \$55.*

SIXTY years ago, few data existed regarding the behaviour and ecology of our closest living relatives, the apes. In 1929 Robert and Ada Yerkes summarized available information in a seminal volume, *The Great Apes*. The intervening years have witnessed rapid growth in laboratory and field studies that have contributed substantially to our understanding of the behavioural ecology of gibbons, orangutans, chimpanzees, bonobos and gorillas. Russell Tuttle ably compiles results of recent research in *Apes of the World*.

Tuttle, following the topical guide provided by the Yerkes, presents readable and succinct accounts of taxonomy, locomotion, feeding, nesting, tool use, cognition, communication and sociobiology. His coverage of each subject is thorough, and his summaries of relevant research are written with care and accuracy. Readers searching for information on virtually every aspect of ape behaviour will be able to find a clear, concise statement in this book. Where empirical studies are currently lacking, Tuttle points constructively to future lines of research. The reference list is extensive, and those wishing to consult the primary literature will find *Apes of the World* an indispensable guide.

The close phylogenetic relationship between apes and humans ensures that the study of ape behavioural ecology will be of particular interest to researchers applying the comparative approach to reconstruct the evolution of human behaviour. Tuttle,

a palaeoanthropologist, has written this book for students and researchers concerned with this problem. Small samples, coupled with reluctance to perform field experiments, limit generalizations about ape behaviour made by primatologists, and models of human evolution based on uncritical acceptance of these generalizations run the risk of oversimplification. Tuttle clearly separates fact from fiction, and his prudent exposition should aid palaeoanthropologists who seek to use results of ape field research to understand human behavioural evolution.

Although Tuttle's lucid treatment is packed with up-to-date information on ape behaviour, *Apes of the World* fails to integrate or to interpret. Perhaps this asks too much. Nevertheless, the vast literature on feeding ecology and social organization, for example, would have been easier to digest with the aid of tables and figures. Similarly, many will be frustrated by Tuttle's limited attempts to place the study of ape behavioural ecology within a broader theoretical and comparative framework. Discussions of fossil evolution, food competition and niche differentiation in the Asian apes and sexual selection in pongids are unsatisfying in the absence of considerations of major theoretical issues such as modes of speciation, the factors influencing ecological diversity and female mate choice. Finally, although some will enjoy Tuttle's light and breezy word-play, others will not be amused by his occasional use of off-colour humour.

These criticisms are minor when compared with the overall quality of this book. Tuttle has written a scholarly work that should serve as a standard reference for many years. With persistent threats endangering apes in their natural habitats, one can only hope that those years will be marked by continuing advances in the study of ape behaviour and ecology. □

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From the outside looking in

Kurt Lambeck

Space Geodesy and Geodynamics. Edited by Allen Joel Anderson and Anny Cazenave. *Academic:1986. Pp.490. £57, \$98.*

THE Earth is subject to a broad spatial and temporal spectrum of deformation, ranging from global plate-tectonic motions over geological time scales of 10^6 years and longer, to the nearly instantaneous and localized deformations associated with surface earthquakes. The evidence for these deformations comes from several complementary disciplines of the Earth sciences — geology, including geomorphology and geochemistry, geophysics and geodesy. The geodetic measurements provide information over a large part of the deformation spectrum, in time from a few hours to decades and longer, and in space from the short lengths of instrumental baselines to intercontinental distances. When interpreted with other geological and geophysical data, the geodetic observations give us valuable insight into the 'instantaneous' deformations and motions of the Earth.

The classical terrestrial observations have contributed greatly to the study of the dynamics of the Earth, as in the geodetic surveys of Japan and New Zealand, and the astrometric observations of the planet's rotation. The so-called space methods of geodesy — the tracking of close Earth satellites and the Moon, and the radio astrometric observations of stellar positions — have a more recent history but they have frequently been held up as a panacea for studies of the Earth's deformation. Results have, however, been slow in coming, and *Space Geodesy and Geodynamics* can be seen as a test of whether the methods can live up to their promises.

The book has four main parts, covering the global gravity field, space geodetic baselines, Earth rotation and dynamics of the lithosphere. Each part contains three or four chapters dealing with selected aspects of the general theme. The part dealing with the Earth's gravity field includes consideration of the results obtained from satellite orbit trajectory analysis, and from surface gravity and radar altimeter data. These observations put constraints upon models of mantle convection and lithospheric rheology and, as such, they contribute to the understanding of the long-term dynamics of the Earth. Several papers on lithospheric deformation partly address questions of lithospheric rheology that may be solved with these data. An omission here is any

discussion of mantle convection, which is an especial pity in view of the significant body of work on seismic mantle topography and its relation to the gravity field.

Another series of papers deals with the measurement of intercontinental baselines. Particularly useful is a paper on the Doppler tracking of satellites, for although the method is itself not very precise, it does provide a fairly uniform and continuous data set over nearly 20 years and we are beginning to see signatures of plate motions in these data. Missing here, unfortunately, is an account of the results from the laser tracking of satellites, but long-baseline interferometric methods are covered; although the data spans are very short, plate tectonic signals can be identified in these data as well. Baseline precision of a few centimetres, over lengths of several thousand kilometres, are being achieved, and it is only a matter of time and money before instantaneous plate motions can be monitored. It may take longer to understand them.

The movement of the Earth's rotation axis relative to the crust and to an inertial reference frame can be followed by a number of the new space methods with much greater accuracy than was previously possible. Several papers discuss

these methods and results: one of the interesting results is the observation of some of the Earth's nutations which have led to the suggestion that the core-mantle boundary may depart from the hydrostatic equilibrium shape. The causes of irregularities in the rotation are considered in three papers, and much of the recent work is reviewed.

Overall, this book presents an up-to-date summary of the status of space geodesy. The individual chapters offer balanced and reasonably detailed accounts of some of the main areas of the subject, and the reader comes away with an appreciation of the results and with a conviction that the long-held promises of the subject are about to be fulfilled. I read the book with a growing awareness of the progress made in the past two decades, and I look forward with excitement to a time when long series of measurements of the Earth's motion and deformation become available. When these are integrated with the geological evidence, we will then have a holistic description of the dynamics of the Earth. □

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Are desert reptiles different?

Eric R. Pianka

Ecophysiology of Desert Reptiles. By S.D. Bradshaw. *Academic:1987. Pp.324. Hbk £29, \$58; pbk £17, \$34.*

THE modest energy requirements of reptiles enable them to capitalize on scant and unpredictable food resources, and these ectothermic animals — especially lizards — flourish in deserts. In his book, Bradshaw reviews aspects of the physiological ecology of certain desert reptiles, particularly two species of Australian agamid lizards, and gives comparative information, drawn from the literature, on some North American and Saharan lizards. The main emphasis is on water and electrolyte regulation, although thermoregulation, growth rates, reproductive tactics and life histories are also considered.

The author sets out to determine whether or not reptiles have evolved any special physiological features that enable them to cope with harsh desert conditions. The book begins with a brief review of the physical characteristics of deserts, followed by short discussions of the historical and biogeographical backgrounds of the herpetofaunae of Australian, North American and the Sahara deserts. The physiological and behavioural bases of lizard thermoregulation are briefly explored, with water and electrolyte balances being examined in some detail.

Lizards can tolerate substantial water losses and body chemistry varies profoundly between species; some, but not all, species possess salt excretory mechanisms. Similarly, reptilian reproductive tactics and life histories are diverse, but no uniquely desert patterns are identifiable. In many species, growth rates are variable between individuals. Case studies spanning three continents (Australia, northern Africa and North America) reveal some similarities, but also certain differences in how two species of agamid and one iguanid cope with life in the desert.

Bradshaw concludes that desert reptiles exhibit no obvious specific adaptations for living in arid places, but argues instead that the reptilian body plan is simply well suited for such environments. His book will be a useful reference for physiological ecologists. □

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• *Temperature Biology of Animals* by A.R. Cossins and K. Bowler deals with animal responses to temperature from the molecular to the whole organism and behavioural levels. The book is just published by Chapman & Hall, price £32.50, \$52.50.



*Incredible blob — a 20-m-high dome fountain of molten lava formed during the eruption of Mauna Ulu, east rift zone of Kilauea Volcano, in October 1969. The dome is held in equilibrium between the upward pressure of magma from below and the mass of lava through which the fountain rises. The picture is taken from the colour original in *Volcanism in Hawaii*, a 1668-page, two-volume set published by the US Government Printing Office, Washington D.C. (US Geological Survey Professional Paper 1350). Volume 1 concentrates on the geology of Hawaii, and Vol. 2 on the structure, dynamics and history of investigations of the area.*

Memories are made of . . . ?

John C. Marshall

Memory and Brain. By Larry R. Squire. Oxford University Press: 1987. Pp. 315. Hbk £25, \$24.95; pbk £13.50, \$14.95.

IN a well-known survey of eighteenth century science Lemuel Gulliver reports the following experiment on the biological basis of memory: "The proposition and demonstration were fairly written on a thin paper, with ink composed of a cephalic tincture. This the student was to swallow upon a fasting stomach, and for three days following eat nothing but bread and water. As the water digested, the tincture mounted to his brain, bearing the proposition along with it." Such swift 'learning by ingestion' continues to intrigue students on the eve of examinations and tourists on the borders of Bulgaria. The notion became scientifically respectable when Hering conjectured in 1870 that the phenomena of memory and of heredity have a common source. And thus the ground was laid for the purported demonstrations in the 1960s that naive flat-worms (*Planaria*) could acquire conditioned reflexes by cannibalizing their trained relatives. Unfortunately, these studies were of dubious replicability and their underlying assumptions were at best a red Hering. But if eating professors is indeed ineffective, what intellectual diet does the academy of Lagado currently recommend?

The last twenty years have seen very substantial advances in the investigation of how memory is represented in the brain. From studies of the biochemistry of synaptic plasticity, through clinico-pathological inquiry into the amnesic syndromes, to information-processing accounts of the functional organization of memory systems, the growth rate of interesting and reliable data has been truly impressive. Larry Squire's account of this work is up-to-date, lucid and comprehensive, with fine sections on the modulation of memory by hormones and neurotransmitter substances, and on the identification of brain structures crucial to (some aspects of) learning and long-term memory in the midline diencephalon and medial temporal region. The importance of these anatomical structures is convincingly demonstrated in patients with stroke, brain ablation, and Wernicke-Korsakoff syndrome, a condition which results from chronic alcohol abuse and involves major retrograde and anterograde amnesia.

In common with many neuroscientists active in this field, Squire has high hopes that "the recent development of an animal

model of human amnesia in the monkey" will resolve some of the anatomical and functional issues that are difficult to address in human cases with very variable pathology. But here I find Squire insufficiently critical. It is far from clear that the profound loss of autobiographical memories characteristic of human amnesia has any analogue in the brain-lesioned monkey's inability to perform delayed nonmatching-to-sample tasks. Whilst animals other than ourselves can clearly learn, it would require more evidence than we possess at present to persuade me that animals have any concept of (specific) memories. Can 'amnesic' monkeys confabulate fictitious autobiographies with the conviction of (some) amnesic patients?

Human patients continue to provide the most revealing data on the fractionation of memory and Squire documents well the surprising dissociations between failure of explicit recall with preservation of skill

learning and tacit memory that are such a striking feature of amnesia. The basic mystery is that these patients behave as if they have learnt from experience yet have no conscious recollection of the experience from which they have veritably learnt; the basic problem is that we are 'data-rich but theory-poor'.

All too often the patterns of memory impairment and preservation have been forced into dichotomous categories labelled by terms vaguely suggestive of different processing strategies or informational types. These labels are no substitute for genuine understanding, and especially so with respect to a system whose functional components are more likely to number two hundred than two. Squire's book provides the data and some useful ways of looking at them. The theory is yet to come. □

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Light reading

R.P.F. Gregory

General Photobiology. By Donat-Peter Hader and Manfred Tevini. Pergamon: 1987. Pp. 323. Hbk £26.95, \$40; pbk £14.95, \$21.95.

THERE is a current upswing of interest in photobiology, as shown by the recent formation of the European Society for Photobiology and the imminent appearance of an associated new journal. The production of this text is therefore timely, both for new undergraduate courses and for newcomers to research in this field. Nevertheless it is a general experience of photobiology societies that the membership shows relatively little interest in the subject as a discipline, and tends to condense into research groups with little or no mutual contact. Given that the only common feature of the subjects under the photobiology umbrella is the absorption of light by primary pigments (bioluminescence is here reclassified as photochemistry), there is a limit to the usefulness of comparison between them: each subject forms more natural liaisons with wider biological fields.

The authors have faced this issue squarely; they have avoided the fragmentation that accompanies multi-author review collections, and have been able to group their topics efficiently. Thus under "Energy Fixation" they assemble bacteriorhodopsin- and chlorophyll-based photosynthesis (the shortest and longest chapters respectively), while photomorphogenesis, light-dependent rhythms, movement responses, vision and the biological effects of UV light are grouped

naturally as "Processes Controlled and Regulated by Light". The depth of coverage is certainly adequate for undergraduate studies, and will provide a stimulus to further study and research.

A second problem recognized by the authors is that biology students often lack a sound physical, chemical and technical background. Roughly a quarter of the text is an introduction to photophysics and photochemistry, introducing units, formulae and the principles and practice of instrumentation. This section comes closest to forging a unity in the subject. A photobiology course including laboratory work requiring both biological observation and proper physical measurement should perhaps become a central requirement in schools of biological sciences.

The presentation of 'physics for biologists' is a difficult educational problem, needing sympathetic teaching. Hader and Tevini's introductory section has some errors and may need reworking in the light of experience. Nevertheless this is a welcome addition to the photobiologist's bookshelf. □

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New in paperback

- *Memoirs of an Unrepentant Field Geologist* by F.J. Pettijohn. Publisher is University of Chicago Press, price is \$10.95, £8.75. For review see *Nature* 312, 224 (1984).
- *Planetary Landscapes* by R. Greeley, with a new chapter on Uranus. Publisher is Unwin Hyman, price is £14.95, \$29.95. For review see *Nature* 320, 664 (1986).
- *Excitons, Selected Chapters*, an abridged version edited by E.I. Rashba and M.D. Sturge. Publisher is North-Holland, price is Dfl.95. For review see *Nature* 307, 572 (1984).

Along-axis variations in seafloor spreading in the MARK area

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Recent investigations with the manned submersible Alvin and the Angus deep-towed camera sled greatly extended the known range of variations in the style of seafloor spreading along the axis of the Mid-Atlantic Ridge. Five transects of the spreading centre at intervals of 10–20 km south of the Kane Fracture Zone at 24° N latitude demonstrate dramatic changes in the style and magnitude of tectonic extension, development of the neovolcanic zone, expression of hydrothermal venting, types of lithologic exposures and morphology of the median valley.

THE MARK (Mid-Atlantic Ridge at Kane) area (Figs 1 and 2) has been the site of many marine geological and geophysical investigations—including dredging^{1–5}, seismic refraction^{6,7}, *Alvin* and *Angus* studies^{8,9}, and recently a site survey for the Ocean Drilling Program (ODP)¹⁰ involving Sea Beam^{11,12}, Sea MARC I¹³, gravity, and magnetics¹⁴ surveys of the rift valley. Subsequent drilling in the median valley has produced a number of shallow holes¹⁰ at sites 648¹⁵, 649¹⁶, and 670^{17,18}. DSDP (Deep Sea Drilling Project) holes 395 and 396 are situated in 7–9-Myr-old crust some 100 km east and west of the rift valley¹⁹. The wealth of information derived from these studies has made the MARK area one of the best known segments of the Mid-Atlantic Ridge and a potential site for a natural laboratory in which to observe seafloor spreading processes.

During June 1986 a multidisciplinary programme of mapping using Sea Beam, *Alvin* and *Angus*, and sampling using *Alvin*, dredges, and hydrocasts investigated along-strike variations in the axial processes in the MARK area. These investigations were concentrated in four transects (Fig. 2) of the ridge axis centred at distances of 30, 45, 60 and 80 km south of the Kane Ridge–Transform Intersection (RTI) and provide geological ground truth in these areas. This area is more than twice the combined lengths of all Mid-Atlantic Ridge segments for which near-bottom geology was previously available.

Transect 1: the Ridge–Transform Intersection

The geology of the RTI area has been described previously⁸, but is reviewed here to illustrate the full range of variations in the area. Seismic refraction studies near the eastern RTI of the Kane Transform^{6,7} show that this is a region of thin (~4–5 km) crust typical of that close to major transforms. The median valley within ~5 km of the RTI (Fig. 3) was found to be very asymmetrical with a much higher (>4 km of relief) and steeper median valley wall on the west compared to the opposing eastern wall (<2 km high). The western wall lies just to the west of the 6,000-m-deep nodal basin and is the site of extensive exposures of metagabbros and greenstones, some of which are derived from coarse-grained cumulate gabbros. Moderate- to low-angle (30–60°) normal faults are truncated by much steeper (70–90°) active faults which suggest substantial crustal stretching^{8,9}. The eastern wall is much more typical of median valley walls

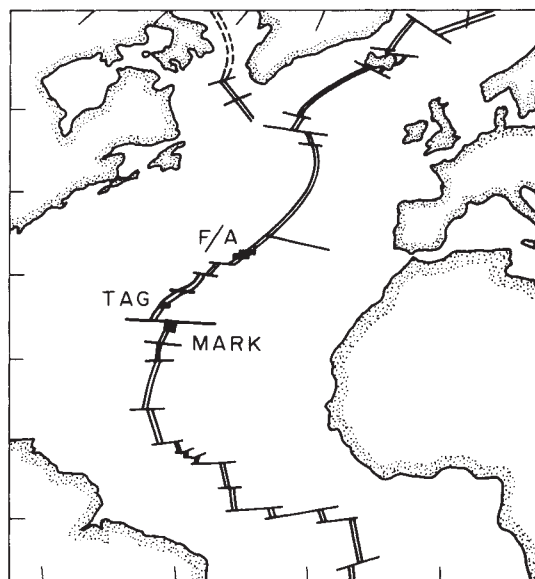


Fig. 1 Location map for the MARK area and other study areas on the Mid-Atlantic Ridge. F/A, FAMOUS and AMAR areas; TAG, Trans-Atlantic Geotraverse.

described elsewhere on the Mid-Atlantic Ridge^{20–23} and is characterized by step-faulted basaltic terrane. The neovolcanic zone, the locus of most recent volcanism, is well defined and transects the trace of the transform to the north where it is defined by a series of volcanic cones built on top of a continuous volcanic ridge. No active hydrothermal venting was found in this region.

Transect 2: centre of an active magmatic cell

Thirty kilometres south of the RTI, the 1986 survey documented the cross-sectional form of the median valley (Fig. 3). Here the rift valley remains somewhat asymmetrical in the same sense as that of the RTI to the north. Greenstones and serpentized

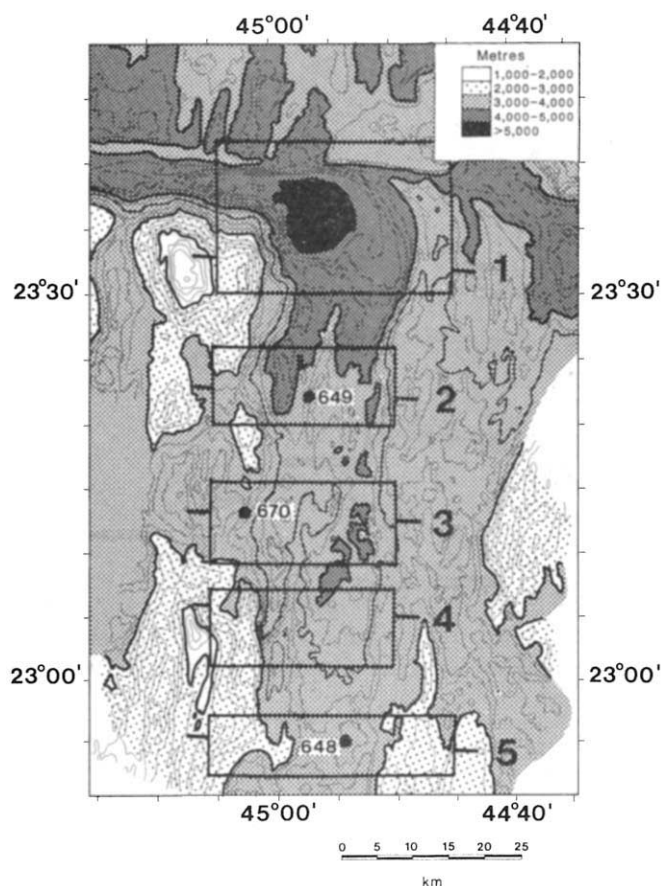


Fig. 2 Generalized Sea Beam bathymetry of the MARK area with the locations of ODP Drill Holes (648, 649, 670) in the median valley. Contours at intervals of 200 m with shaded areas between even 1,000-m contours. Note the nodal basin at the Kane RTI is outlined by the 5,000-m contour and the median valley is approximately outlined by the 3,600-m contour (bold). Transect areas 1-5 and cross-section locations (bars) are indicated.

peridotites were recovered from the western median valley wall where moderately dipping faults and younger, active high-angle faults were again present. The opposing eastern wall yielded only basaltic rocks from a block-faulted terrane similar to that observed in transect 1. The neovolcanic zone coincides with the crest of a 600-m-high, linear, volcanic edifice located near the axis of the median valley. This neovolcanic ridge is continuous over at least 40 km along the median valley axis, and is by far the largest single volcanic constructional feature ever found on the Mid-Atlantic Ridge. It extends southwards from the neovolcanic zone of the RTI but becomes progressively older-looking south of transect 2 where basalts of the ridge become more fractured and covered with pelagic ooze. The Snake Pit hydrothermal area lies at the crest of the neovolcanic ridge near the centre of this transect. This active vent field consists of more than 12 separate black-smoker chimneys in a field of inactive vents and vent deposits^{16,24,25} covering at least 0.16 km².

This vent field was initially located during the ODP site survey¹⁵ and was subsequently drilled and photographed by the scientists aboard the RV *Joides Resolution* at Site 649¹⁶. During the 1986 cruise, hydrothermal fluids and deposits, vent fauna and hydrothermally altered basalts were collected. The active vents and water samples obtained from them proved to be remarkably similar to those of the East Pacific Rise. Silica concentrations in vent fluids (<2% sea water) reached levels of 17.5 mM kg⁻¹ implying a depth of reaction of ~1.7 km (ref. 26). The fluid compositions and temperatures (~350 °C) strongly

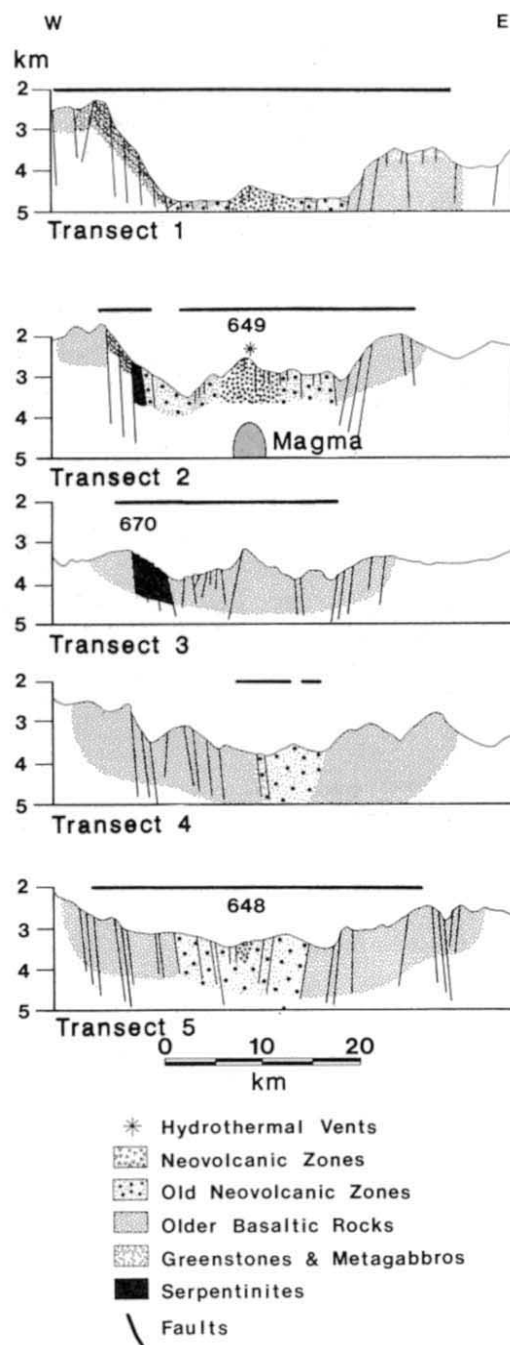


Fig. 3 Schematic geological cross-sections of transects in the MARK area. Solid line indicates areas constrained by *Alvin* and *Angus* data. See Fig. 2 for locations. Bold lines show prominent high-angle faults, inferred to be straight at depth. (Note that significant extension may occur on low-angle faults that are not shown.) Neovolcanic zone—youngest extrusives; Old neovolcanic zone—extrusives with light sediment cover; Older basaltic rocks—weathered extrusives with heavy sediment cover. Vertical exaggeration: $\times 3$.

suggest the presence of a magma chamber ~2 km deep in the crust. Hydrocasts in this area showed large manganese anomalies (5–10 times greater than background levels) centred at depths of ~3,500 m, implying the presence of additional active vents at greater water depths than those of the Snake Pit. Seismic refraction results from ~5 km east of the neovolcanic ridge indicate a crustal thickness of ~5 km with normal mantle velocities⁷, hence limiting the half-width of the area occupied by any axially symmetric magma chamber(s) to ≤ 5 km. Basaltic

rocks sampled all along this ridge are normal mid-ocean-ridge basalts (MORBs) with a limited compositional range^{4,27}.

Transect 3: a zero-offset transform

At 45 km south of the RTI the Mid-Atlantic Ridge axis is radically different from any other segment yet surveyed (Fig. 3). One of the most obvious anomalies at this latitude is the lack of a well-developed median valley (Fig. 2). Rugged bathymetry with ridge axis-parallel ridges and valleys occurs across the trace of the median valley segments to the north and south. Fault-bounded blocks, some of which are up to 400 m high, occur across the entire area. The blocks are asymmetrical with steep, faulted faces to one side and less steep, back-tilted lava flow surfaces to the other. The valleys between the blocks are intensely fissured, and blanketed by several tens of centimetres to perhaps one metre of pelagic sediment. There is no typical neovolcanic zone in this segment of the ridge axis. The neovolcanic ridge extending southwards from transect 2 becomes more subdued and increasingly faulted and covered by pelagic sediment before it enters transect 3. Hydrocasts show very low concentrations of manganese in bottom waters indicating an absence of vigorous hydrothermal activity. Some ferromanganese-coated, but relatively fresh-looking, basaltic rocks were recovered from a line of four or five conical volcanoes that lie along the trace of the eastern median valley walls. Although these volcanoes are not extensively faulted and fissured, heavy sediment cover indicates that they are several tens of thousands of years old.

Serpentinized peridotites were recovered from a wide (>1 km) region on the western edge of the area. Subsequent drilling by the RV *Joides Resolution* penetrated ~100 m of serpentinite at this locality^{17,18}. The serpentinites show petrographic features that indicate a protracted deformation and alteration history including high-temperature, ductile deformation and subsequent hydration at progressively lower temperatures²⁸. Part of the high-temperature deformation appears to have occurred at very high differential stresses and may be related to the type of upper mantle seismicity documented beneath the median valley floor just south of the present study area²⁹.

The seismic velocity structure beneath this area is also anomalous in that seismic energy does not propagate through the crust and low upper mantle velocities were recorded⁷. These characteristics may be due to intense fracturing and serpentinization beneath the spreading centre.

The geological expression of the spreading centre in this area is like no other segment of the Mid-Atlantic Ridge studied to date. Its position just south of the neovolcanic ridge (Fig. 4) and its lack of youthful volcanic features suggest that it may be regarded as the boundary between two magmatic cells⁷ or a 'zero-offset transform'³⁰. The only features observed that could be related to strike-slip faulting are a family of north-west trending (295°) fractures in the northern part of the transect.

Transect 4: symmetrical valley/volcanic cones

A relatively small amount of information on near-bottom geological conditions was collected in transect 4 (Fig. 3), but the geology of the spreading centre is constrained by these observations. Sea Beam bathymetry (Fig. 2) shows a well-defined, symmetrical median valley crossed obliquely by lineaments defined by lines of small conical volcanoes (Fig. 4). These volcanoes appear to be built upon a relatively old, fissured and sediment-covered swath of lavas that floor the median valley. As in transect 3, no youthful neovolcanic zone was found. The hills that were visited with *Alvin* or photographed by *Angus* were faulted and had substantial sediment covers, of up to several tens of centimetres thick, on gently inclined surfaces. Samples collected by *Alvin* or by dredging were relatively weathered and had thin surface coatings of ferromanganese oxides. The median valley walls were not studied extensively in this area, but laterally continuous, linear scarps spaced at 5–7 km probably represent

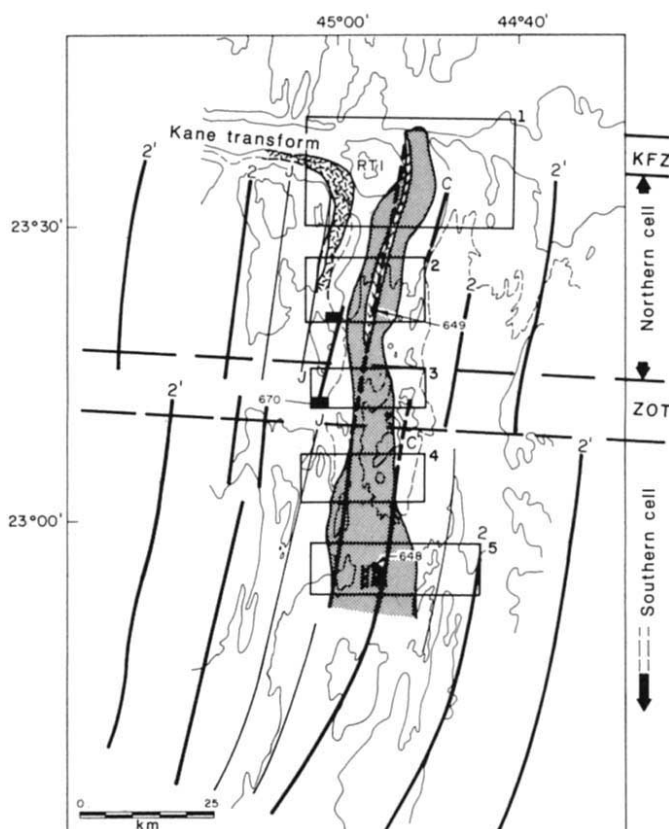


Fig. 4 Summary tectonic map of the MARK area and surrounding seafloor. Symbols: black—serpentinite, dashes—greenstone and metagabbros, v's—neovolcanic zone, stippled—old neovolcanic zone, bold lines—magnetic anomalies^{10,14} (2', 2, J—Jaramillo, C—central anomaly); contour interval 1,000 m and 3,600 m (dashed). Two spreading cells are separated by a zero-offset transform (ZOT) at transect 3. Note that part of the central magnetic anomaly may be accounted for by exposed serpentinites on the western median valley wall.

active fault zones^{10,13}.

This area appears to be one in which a sequence of faulting and volcanism typical of that observed elsewhere on the Mid-Atlantic Ridge has taken place. The last major volcanic construction in the area was probably at least ten thousand years ago, but as in the ALVIN Mid-Atlantic Ridge (AMAR)^{22,31,32}, small volumes of magma may have leaked into the crust more recently. Transect 4 might be expected to become more volcanically active after the present interval of non-volcanic tectonic extension.

Transect 5: regional axial bathymetric high

This transect is located at ~80 km south of the Kane RTI (Fig. 3) and resembles transect 4 just to the north. The median valley walls are symmetrical and made up of evenly spaced (~5 km) linear fault scarps. Where visited, these fault scarps exposed only basaltic rocks and, with the exception of only a few areas, appeared to be tectonically inactive. Debris-slide accumulations, however, may have obscured signs of recent movements. The centre of the median valley is marked by a recognizable neovolcanic zone but, as in transect 4, most of the lavas are considerably older than those of the neovolcanic zone in the northern two transects. However, 5 km south of the Serocki volcano, three patches of relatively fresh volcanics, each ~1 km wide, were found. A dredge haul in one of these areas recovered the freshest basalt yet sampled in the MARK area. Well-formed conical volcanoes lie along lineaments that trend north-north-east and west-north-west and intersect at the regional bathymetric high near Serocki volcano, ODP Site 648 (Fig. 4). Serocki volcano proved to be a relatively old feature with numerous faults and

fissures and nearly continuous sediment cover on areas of low relief³³. Several other smaller volcanic cones in the vicinity showed similar morphological characteristics, but some yielded relatively fresh lava suggesting relatively young but small volcanic eruptions.

By analogy with the systematic relationships found on the East Pacific Rise³⁴, the regional bathymetric high was predicted to be the site of the most recent magmatism and vigorous hydrothermal activity in the MARK area. Seismic refraction studies in this region indicated a lower crustal low-velocity zone suggesting a magma chamber or a body of recently cooled, cracked rock not yet healed by hydrothermal mineral growth⁷. Despite all expectations, no signs of active vents and only limited exposures of young lavas were found there. Given the amount of time devoted to the study of this area and the difficulty in locating other vents on the Mid-Atlantic Ridge, the possibility of active vents in this area cannot be ruled out. This part of the median valley appears to be the centre of a relatively inactive magmatic cell that extends northwards through transect 4 and southwards for an unknown distance.

Along-axis variations

From the descriptions and interpretations above it is obvious that dramatic variations in the geological character of the median valley occur over the 80-km length of ridge axis surveyed south of the RTI. These differences may be due to diachronous development of tectonic extension^{35,36} and volcanism along the axis or perhaps to persistent differences in the style of seafloor spreading from cell to cell along the spreading centre, or both. Whereas cyclic processes have been invoked to account for differences between different ridge segments elsewhere^{21,37,38}, these segments are separated by well-defined transform faults.

The present configuration (Fig. 4) appears to consist of a magmatically active spreading cell ~40 km in length that extends from the RTI through transect 2. A much older, magmatically inactive cell appears to extend through transects 4 and 5. The anomalous region in transect 3 that separates these two areas may be a ridge segment that has been magma-starved during the evolution of the two adjacent segments. These three segments have distinct morphologies, lithologic associations, structures, and geophysical characteristics.

The magnitude of tectonic extension, reflected in the fault structures, appears to vary significantly along the axis. The stretching and thinning of the crust have been extreme in the northern three transects, but appear to be less important in the southern two transects, at least for the last cycle of spreading represented by the geology of the median valley. The crust of the northern cell has apparently suffered sufficient tectonic extension and thinning of the shallow-level volcanic units to expose mafic and even ultramafic plutonic rocks. Whereas serpentinite diapirism may have played a role in exposing the ultramafic rocks^{39,40}, this mechanism cannot explain the exposure of coarse-grained cumulate gabbros that probably crystallized at depths >2 km. If faulting has exposed these deep-level gabbroic rocks, then it seems likely that faulting is also the primary cause of the exposure of the nearby serpentinites. These areas may be regarded as submarine analogues of the highly extended terranes of the Basin and Range Province of the western United States⁴¹ where extreme crustal stretching and thinning have exposed relatively deep-level rocks.

Volcanism has taken a very different form in the two spreading cells. In the northern cell, robust magmatism has led to an extremely large constructional volcanic ridge (4 km × 40 km × 600 m). This is much larger than volcanic constructional features observed in the neovolcanic zone of the FAMOUS area (1 km × 2 km × 150 m)⁴⁰. In the southern cell, volcanism has taken the form of small (≤1–2 km diameter × 100 m high) volcanic cones, in some cases with small summit craters. In some instances, two or three cones have coalesced to form elongate ridges 3–4 km long and 100–200 m high resembling those of the FAMOUS

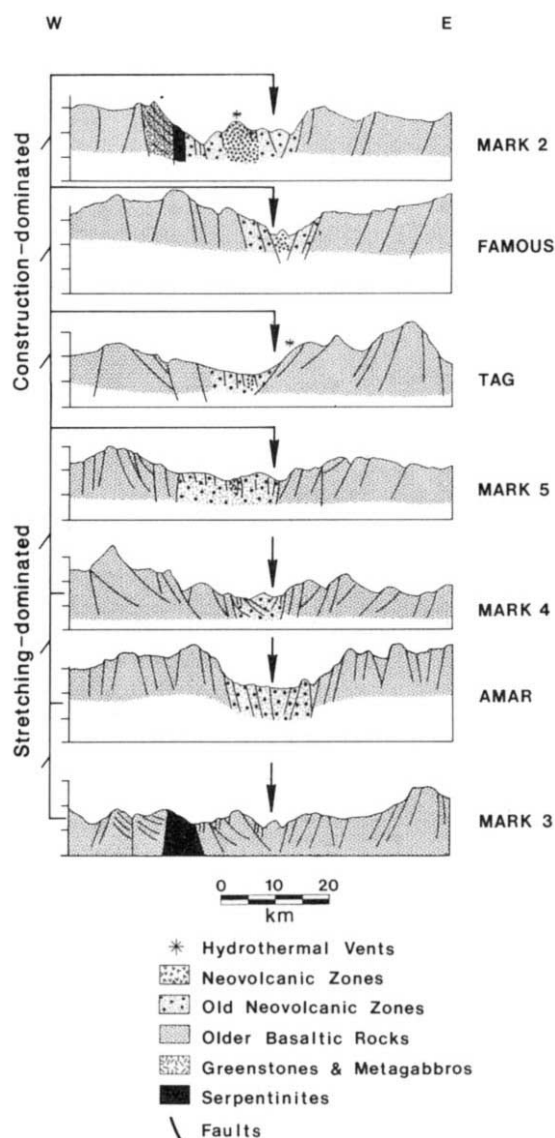


Fig. 5 Flow chart for median valley evolution along the Mid-Atlantic Ridge (see text for explanation). Geometry of faults at depth is schematic. Vertical exaggeration: ×3. Cross-sections based on data from FAMOUS/AMAR (ref. 22), TAG (ref. 23 and P. Rona and G. Eberhart, personal communication, 1986, TAG) and this study.

area. The neovolcanic ridge in the northern cell, in spite of its large volume and observations of at least six or seven different flow units each ≤100 m thick, has a limited compositional range that is distinctive from other magmatic units in the MARK area²⁷. This is consistent with the presence of a single, relatively large magma chamber beneath the ridge. The much smaller volcanoes of the southern cell, like Serocki Volcano, each has a very limited compositional range that is distinct from those of surrounding volcanoes. Here, either the magma chambers that fed them are small and short-lived or are being tapped at different levels or different stages of evolution.

These observations raise important questions regarding the nature of seafloor spreading on the Mid-Atlantic Ridge. How stable are these spreading cells in terms of their relative positions, dimensions, and processes? Does the anomalous region in transect 3 lie at a point that persistently defines the boundary between two spreading cells? If this is a stable configuration, such a segment of the ridge axis may produce a different type

of lithosphere than that produced near the centres of spreading cells as, for example, in transect 2. Lithosphere produced in a spreading cell similar to that in transect 2, which has been greatly stretched between volcanic constructional episodes, may in turn differ significantly from that produced in cells with less stretched lithosphere and more continuous magmatic leaking, as in transects 4 and 5. This type of variation would be consistent with models of lithosphere 'ribbons' that form along seafloor spreading flow lines^{30,43-45}.

The magnetic anomaly patterns along flow lines from this area (Fig. 4) show lateral offsets of 10–15 km for anomalies 2 and 2', but after a series of small ridge-axis jumps, the central anomaly is now offset by less than the width of the median valley in transect 3^{10,14}. Thus although the spreading-centre offset at this latitude has been variable over the past 3 Myr, at present there appears to be only a very small-offset (<8 km) or essentially 'zero-offset transform'. The anomaly patterns suggest that the cells are stable, but that the offset between them has changed during the last 3 Myr.

Seafloor spreading on Mid-Atlantic Ridge

Surveys of the FAMOUS and AMAR areas have led to the suggestion that these two areas represent two different stages of a cyclic process of seafloor spreading^{22,31,32,46}. In this view the FAMOUS Rift Valley represents an area that has had a relatively recent volcanic constructional event. In contrast, the AMAR Rift Valley, which has only a very narrow neovolcanic zone and is much more faulted and fissured, represents an area in which tectonic extension dominates. These different median valley structures have been interpreted as end-members of a cyclic process in which the median valley alternates between these two forms with more or less continuous tectonic extension and periodic magmatism. The new results from the MARK area greatly extend the extremes of any cyclic process that operates on slow-spreading ridges. Some possible cycles for individual spreading cells are outlined in Fig. 5 in the form of a flow chart. The arrows connect cross-sections of the various Mid-Atlantic Ridge segments for which there are sufficient geological data to develop a comparison. In this scheme, the areas that lie near ridge-transform intersections have been omitted because they lie in somewhat different thermal and mechanical settings from the rift valley segments discussed here.

In the flow chart, MARK transect 2 is at the top and represents the area with the most robust neovolcanic zone. At present,

volcanic construction dominates this area. Below, the other Mid-Atlantic Ridge cross-sections are arranged in order of decreasing width and definition of the neovolcanic zone and increasing development of extensional tectonic features. MARK transect 3 lies at the bottom of the chart, and the FAMOUS and AMAR rifts lie just inside the top and bottom profiles (Fig. 5). If the spreading cells are stable through time as described above, then crustal processes at various points on the ridge axis may be described by a loop in the flow chart. For the FAMOUS and AMAR areas a loop that completes a circuit between their present structures may apply. For a segment similar to MARK transect 2, where extreme stretching resulting in the exposure of plutonic rocks, and voluminous volcanism producing a robust neovolcanic ridge alternate, the full circuit may apply. MARK transects 4 and 5 appear to have magmatic construction keeping pace with extension by the leaking of relatively small volumes of magma into the crust without large amounts of stretching. A zero-offset transform or cell boundary may be described by a loop near the bottom of the chart in which only minor magmatic additions occur during continuous, and possibly extreme, crustal stretching. This can result in locally large magnitudes of crustal thinning.

Such a scheme can account for a large number of ridge-axis morphologies and different cyclic behaviours. But this model applies only if the cells are stable through time. Shortened or extended circuits in the flow chart may occur if instabilities, such as propagations, develop. Because the full range of variations in the style of spreading for slow-spreading ridges probably has not yet been observed, this flow chart will need to be modified as more data become available. Given the available information, we believe that this is a useful way in which to consider the along-axis variations in seafloor spreading on the Mid-Atlantic Ridge. Whereas previous studies have emphasized that magmatism and tectonic extension are only out of phase in different spreading cells, data collected in this study suggest that these two processes have different relative importance in different cells, possibly producing ribbons of different types of oceanic lithosphere.

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The *Drosophila* clock gene *per* affects intercellular junctional communication

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The per locus of Drosophila has been implicated in the control of behavioural rhythms. In fruitfly embryos and larvae per is expressed in salivary glands. Per mutations have striking effects on intercellular communication in salivary glands: gap junction channels are modulated so that their conductance varies inversely with the period of behavioural rhythms in the mutants. A similar effect on junctional communication in the nervous system may explain how per influences behavioural rhythms.

RHYTHMIC oscillations in behavioural and physiological processes are generally thought to reflect the function of an endogenous pacemaker or biological clock. Pacemakers have been located in discrete neural structures, including the mammalian suprachiasmatic nucleus, the avian pineal gland and the *Aplysia* eye (see ref. 1 for review). Rhythms generated by these structures are thought to originate in networks of tonically active cells. These serve as central oscillators, thought to be linked by neural or hormonal circuits to overt biological processes exhibiting circadian or shorter period (ultradian) rhythms.

Mutations that alter the expression of rhythmicity have been described in *Drosophila*, *Chlamydomonas*, and *Neurospora*². In *Drosophila*, changes in the structure or abundance of the *per* gene product can lengthen, shorten or abolish periodicity of circadian and ultradian behavioural rhythms^{3,4}. DNA sequencing and preliminary biochemical analyses show that the *per* protein may be a proteoglycan⁵⁻¹².

Analyses of flies that are genetic mosaics for *per* have demonstrated that the tissue focus for its effect on circadian locomotor rhythms lies in neural tissues in the head of the adult¹³. In contrast, an ultradian rhythm detected in male courtship song maps to thoracic tissues, presumably the thoracic ganglia¹⁴. Mutations at the *per* locus also affect the expression of rhythms in larval tissues; *per*⁰ alters the periodicity of larval heartbeat (M. S. Livingstone, personal communication) and the circadian uptake of a voltage-sensitive dye by isolated salivary glands¹⁵.

Although these observations led to the suggestion that *per* is directly involved in the formation of a central oscillator, two properties of *per* mutants indicate that it may function as a 'coupler' which synchronizes or organizes clock components. First, circadian rhythms are found in some cells in explanted salivary glands from *per*⁰ (behaviourally arrhythmic) individuals¹⁵; second, ultradian rhythms are often observed in the locomotor activity records of flies lacking a functional *per* locus¹⁶.

In this report we examine the distribution of *per* RNA and protein in embryos and selected larval tissue. RNA from the *per* locus and its protein product are expressed abundantly in the salivary glands of embryos, and *per* protein is also expressed in the salivary glands of third instar larvae. Measurements of dye permeability and electrical coupling in salivary glands of *per* mutants indicate that the gene is central in intercellular communication. We propose that *per* affects behavioural rhythms by altering gap junctional communication in the nervous system of *Drosophila*.

Localization of *per* products

Figure 1a-d illustrates the results of *in situ* hybridization to sectioned 10- to 20-h embryos, using ³⁵S-labelled RNA probes

homologous to untranslated regions in the 5' and 3' ends of the *per* transcription unit. These probes have been shown to hybridize only to *per* transcripts and include sequences homologous to certain rare, variant transcripts¹². Following hybridization, cells in the developing salivary gland are strongly labelled in all embryonic stages older than 10 h. Little or no labelling is apparent above background levels in other tissues throughout embryonic development.

Male flies hemizygous for the deficiency *Df(1)64j4* are viable throughout embryogenesis¹⁷ and lack the DNA sequence encoding the *per* locus^{5,6}. Large numbers of exclusively male embryos can be produced by crossing *Df(1)64j4/Df(1)TEM202* females (*Df(1)TEM202* is also *per*⁻) to males carrying the meiotic drive system *Segregation Distorter* on their Y chromosomes (ref. 18; Fig. 1, legend). *In situ* hybridization to these sectioned embryos with identical probes fails to label salivary glands (data not shown). Thus, it would appear that salivary glands in embryos are the major source of the *per* transcript.

The developmental expression of *per* transcript is illustrated in Fig. 2. During embryogenesis, the 4.5-kilobase (kb) transcript can be visualized first in 8- to 12-h embryos and persists through development with peak abundance in 12- to 16-h embryos, and subsequently, in pupae and adults. The embryonic expression parallels the ontogeny of salivary gland development¹⁹. Salivary glands are first seen as ectodermal invaginations in late stage-11 embryos (~7 h) and begin to display signs of secretory activity from stage 13 (~10 h) onwards.

The *in situ* results presented here differ from an earlier report by James *et al.*²⁰. They reported very weak hybridization in the region of the ventral nerve cord in 10- to 18-h embryos, but did not report hybridization to the salivary glands.

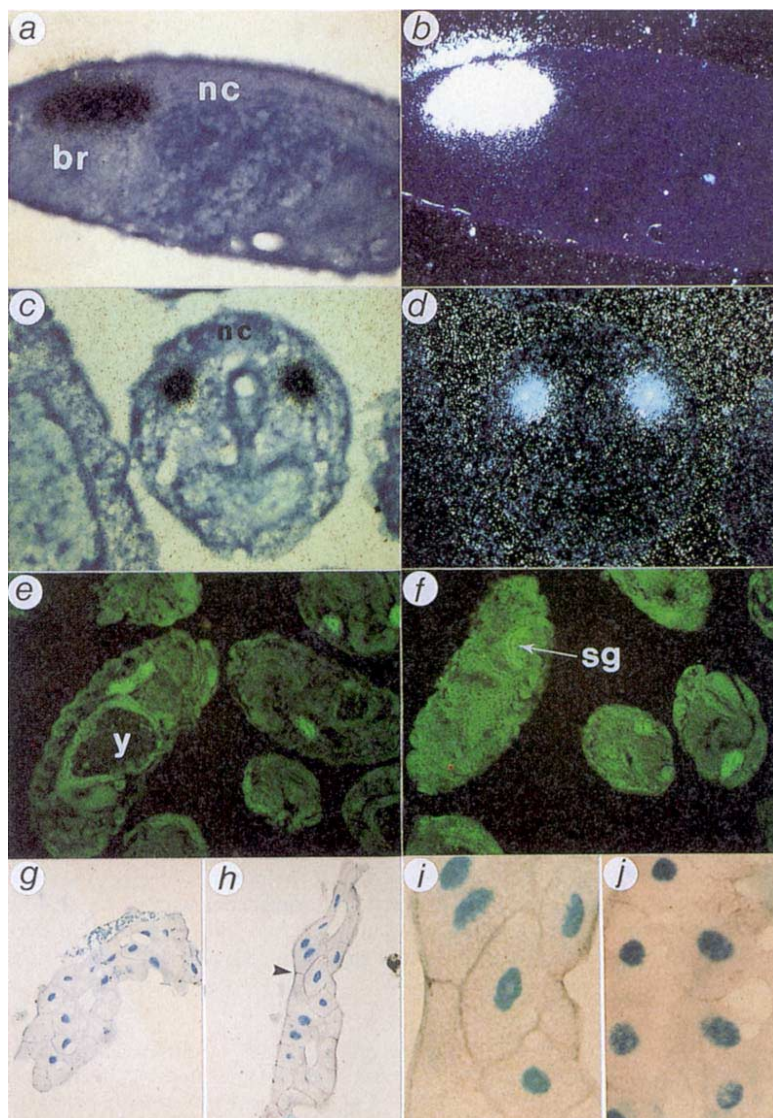
Embryonic salivary glands are also labelled (Fig. 1e-f) by indirect immunofluorescence using a primary antibody directed against a synthetic *per* peptide. This peptide corresponds to a series of amino acids at the carboxy terminus of the predicted *per* protein (ref. 10; Fig. 1, legend). The developmental expression of this antigen in embryonic salivary glands closely parallels that described with RNA probes (Fig. 1, legend). In 10 to 12-h sectioned embryos, the *per* product seems to be at cell boundaries and on the cell surface. Similar results are seen with sectioned glands isolated from third-instar larvae, using a horseradish peroxidase conjugated second antibody (Fig. 1h, i). No labelling of cell boundaries is apparent in salivary glands isolated from *Df(1)TEM202/Df(1)64j4* larvae (Fig. 1j).

Biochemical characterization of the *per* protein

DNA sequence homologies have been reported between genes encoding well-characterized proteoglycans and *per* (refs 9 and 10). Preliminary descriptions of the *per* gene product indicate that it may have the characteristics of a heparan sulphate proteoglycan¹¹. The antibody used in our *in situ* localization was

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Fig. 1 Distribution of *per* gene products. *a-d*, *In situ* hybridization of ^{35}S -labelled riboprobes to 10- to 12-h (stage-14) sectioned embryos. DNA templates transcribed correspond to restriction fragments a8 and d1 described in Fig. 1 of ref. 10. *a, b*, Bright-field and dark-field photographs of the same sagittally sectioned embryo (2-day exposure); *c, d*, transverse section through the anterior portion of an embryo (5-day exposure). *Per* transcripts are detected in the developing salivary gland. Similar patterns of expression are evident in embryos at 10–24 h. No signal is apparent in embryos younger than 10 h, nor were signals readily detected in other tissues on long exposures (10–15 days). No signals were detected in male embryos hemizygous for *Df(1)64j4*, or in wild-type embryos with riboprobes synthesizing the sense strand. *e, f*, Localization of *per* protein in sections of 10- to 12-h embryos using antibodies against *per* product visualized with fluorescein-conjugated goat anti-rabbit antibodies (Jackson Labs). These antibodies react strongly with cells in salivary glands of embryos older than 10 h. The expression of *per* protein in salivary glands is not restricted to embryos. *g-j*, Sections from isolated third-instar larval salivary glands, treated with antibody against *per* protein visualized with horseradish peroxidase (HRP)-conjugated second antibody. The cell nuclei in these sections were visualized by counterstaining with Giemsa. *g*, Wild-type salivary gland stained for HRP in the absence of primary antibody but following treatment with second antibody. *h, i*, In wild-type glands, *per* proteins are located on the cell surface and the intercellular membranes. Arrowhead in *h*, region shown at higher magnification in *i*. *j*, Antibodies were applied to *per*⁻ (*Df(1)TEM202/Df(1)64j4*) sectioned glands. Note the absence of staining on intercellular membranes (compare *i*). Abbreviations: br, brain; nc, nerve cord; sg, salivary gland.



Methods. Tissue sections were prepared as follows: staged embryos were dechorionated in freshly prepared 50% chlorox for 3 min and fixed in a solution of 5 ml heptane, 2.5 ml 8% paraformaldehyde, 2 ml of dimethylsulphoxide (DMSO) and 0.5 ml of 1X phosphate buffered saline (PBS) for 15 min with constant agitation. The vitelline membrane was removed in heptane-methanol-EGTA³² and embryos embedded in OCT (Miles Scientific) on dry ice. Sections (8 μm) were post-fixed in 4% paraformaldehyde in PBS for 15 min and prepared for *in situ* hybridization as described³³. Hybridization and wash conditions were as in ref. 34. Cryostat sections for immunocytochemistry were fixed in 2% paraformaldehyde in PBS for 15 min. After washing in PBS, sections were blocked with 5% goat serum, and incubated for 14 h in a humidified chamber with affinity purified antibodies against *per* product in 5% goat serum/PBS. Regions of primary antibody localization were visualized with fluorescein-conjugated goat anti-rabbit immunoglobulin (IgG, Jackson Labs) diluted 1/500 with 5% goat serum in PBS, or using a HRP-conjugated anti-rabbit IgG supplied by Vector Lab (Vectastain ABC kit). A peptide corresponding to amino-acid residues 1,095–1,110 (ref. 10) was synthesized and coupled to keyhole limpet haemocyanin (KLH) and used to immunize two separate rabbits by standard procedures. Antibodies directed against the peptide were purified by passing rabbit sera over a 5-ml affinity column containing 1 mg of the synthetic peptide covalently linked to Affigel 10 resin. Bound proteins were extensively washed with 1 M NaCl and eluted in acetic acid, pH 2.5, neutralized with 1.0 M HEPES, pH 9.0, and dialysed against PBS. Antibody titre determined by enzyme-linked immunosorbent assay (ELISA) was in excess of 1:25,000 following affinity purification. No reactivity to KLH could be detected. The affinity purified antibodies against *per* product also recognize the epitope in *per* fusion proteins (see Fig. 3). Male embryos that were *per*⁻ were produced by crossing *T(Y;2)SD-72, T22/cn bw* (ref. 18) males to *Df(1)64j4/Df(1)TEM202* (*per*⁻) females. No appreciable development occurs in female offspring, as they are aneuploid for most of chromosome arm 2L. By contrast *Df(1)64j4* males survive throughout embryogenesis¹⁷.

immobilized on Affigel 10 beads (BioRad) and used to purify antigen from protein extracts of adult flies. This procedure results in the isolation of a large molecule which cannot be resolved on polyacrylamide gels (Fig. 3A). Digestions of the high relative molecular mass (M_r) preparation with enzymes that remove carbohydrate side chains (chondroitinase ABC, heparinase, or keratanase) have no major effect on protein mobility as determined by polyacrylamide gel electrophoresis (PAGE). Following digestion with heparinase II, a polydisperse band with an average M_r of 100,000 (100K) can be resolved (Fig. 3B). This size is approximately that expected for the *per* product^{10,12}. Reddy *et al.*¹¹ have reported that an unpurified antigen is sensitive to heparitinase digestion by virtue of its behaviour on DEAE-Sephacel columns. From the combined

studies, we conclude that the *per* product behaves as a proteoglycan, but that the glycosaminoglycan chains may involve structures other than simple heparan sulphate linkages.

Initial characterization of protein preparations from salivary glands has indicated that antigen with proteoglycan properties can be immunoprecipitated from wild-type but not *per*⁰ glands (T.A.B., unpublished data). The absence of antigen in *per*⁰ is expected because DNA sequence analysis of this mutation predicts that *per*⁰ flies produce a truncated protein about one third of the size of the normal gene product^{3,4} and lack the epitope recognized by the antibody employed. The *per* protein appears to be a proteoglycan, and our localizations suggest that it may form part of the extracellular matrix at cell boundaries in *Drosophila* salivary glands.

a b c d e f g h i j k l m

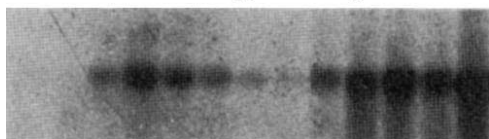


Fig. 2 Developmental analysis of *per* transcripts. A ^{32}P -labelled riboprobe was produced from *per* restriction fragment a8 as in Fig. 1. The probe detects 4.5-kb *per* transcripts in all stages except 0–8-h embryos. *Drosophila* embryos, larvae and adults were allowed to develop at 25 °C. Poly(A)⁺ RNA (5 µg) from embryos at, lane a, 0–4 h; b, 4–8 h; c, 8–12 h; d, 12–16 h; e, 16–20 h; or from first instar (lane f), second instar (g) and third instar (h) larvae; or from day old (lane i) 2-day (j), 3-day (k) and 4-day (l) pupae; or from adults (lane m), was separated on a 0.8% agarose, 2.2 M formaldehyde gel and transferred to a membrane. Hybridization and wash conditions were as described⁸. The panel was overexposed to demonstrate expression of *per* transcripts in second and third larval instars, and lack of detectable expression in 0–8-h embryos.

Per mutations alter intercellular communication

That the *per* gene product is expressed in salivary glands is not entirely surprising. Rensing and coworkers¹⁵ have reported the existence of circadian rhythms in isolated salivary glands using a fluorescent dye, 3,3'-dihexyloxycarbocyanine iodide. This compound belongs to a class of slow, voltage-dependent, membrane-permeable dyes which are useful in measuring periodic changes in electrical potential across biological membranes (see ref. 21 for review). Circadian rhythms in fluorescence were reported for most cells in wild-type glands. In *per*⁰ flies, significantly fewer cells were rhythmic, and rhythmic cells showed

a decreased amplitude in fluorescent intensity¹⁵. One possible explanation for the observed difference is that cells behave asynchronously in *per* mutants; that is, within a *per*⁰ gland individual cells differ in the magnitude of changes in electrical potential across their membranes, as measured by their ability to concentrate the fluorescent dye. Insect salivary glands are expected to behave as a syncytium, as a consequence of intercellular coupling mediated by gap junctions (see ref. 22 for review). The lack of coordinated function in *per*⁰ salivary glands suggested that cell communication might be altered in *per*⁰ mutants.

The synthesis and function of gap junctions in primary liver cell cultures are modulated by the presence of proteoglycans in the culture medium²³. Thus, it seemed possible that the *per* gene product has a similar function in salivary gland junctional communication.

Cell communication in *per* mutants and wild-type flies was examined by a series of dye coupling experiments using Lucifer Yellow and quantified by electrophysiological recordings which measured electrical coupling between cells in salivary glands. Lucifer Yellow diffuses readily between cells in salivary glands from wild-type third instar larvae, but is virtually confined to the injected cell in salivary glands isolated from *per*⁰ (Fig. 4). This difference in diffusion is not due to developmental variability in cell coupling because salivary glands are dye-coupled throughout the third instar larval stage, and dye transfer is also evident in some cells of wild-type late second instars (data not shown). Little or no coupling could be detected in salivary gland preparations from three *per*⁰ strains (*per*⁰ *w spl*; *per*⁰ *sn m*; *y per*⁰, *ry*⁴²; at least five independent salivary gland preparations were examined from each strain). In addition, salivary gland cells from a transformed strain, *y per*⁰, *ry*⁴², P1/25, display more extensive dye transfer (data not shown). This strain has long-period behavioural rhythms, expresses a fivefold reduction in the amount of the 4.5-kb *per* transcript relative to wild-type flies, and differs from the *y per*⁰, *ry*⁴² strain only by virtue of the

Fig. 3 Autoradiograms of ^{125}I -labelled proteins from *D. melanogaster* adults and extracts from *Escherichia coli* expressing *trpE-per* fusion proteins separated by SDS-polyacrylamide gel electrophoresis (PAGE). Lane a, *D. melanogaster* proteins purified by affinity chromatography; lane b, same as lane a, but following digestion with heparinase II, a protein of *M*_r 100,000 (100K) is bound to the affinity matrix and resolved on SDS-PAGE; lane c, immunoprecipitation of fusion proteins expressed in *E. coli* using the same affinity purified antibody as in lanes a and b; lanes d and e, same as lane c, but using pre-immune sera (lane d) or antibody against *per* protein plus 1 µg of the synthetic peptide antigen as a competitor (lane e). Comparison of lanes c–e indicates that antibodies directed against the 16 amino-acid synthetic *per* peptide recognize the epitope in a *M*_r 68K fusion protein. The fusion protein contains 32K of the C terminus of the predicted *per* product.

Methods. Protein extracts were prepared from frozen, pulverized *D. melanogaster* adults by extraction for 48 h at 4 °C in a solution containing 4 M guanidine HCl, 50 mM sodium acetate, pH 6.0, and protease inhibitors (5 mM EDTA, 10 mM benzamide HCl, 10 mM *N*-ethylmaleimide, 10 mM aminocaproic acid, 1 mM phenylmethylsulphonyl fluoride (PMSF), 0.1 mM pepstatin A) with constant stirring. Extracts were dialysed five times with buffer containing 7 M urea, 0.1 M Tris, pH 7.0, and protease inhibitors, and applied to a 50-ml column of DEAE-Sephacel equilibrated with the same buffer. The column was washed with 10 vols of application buffer, and 20 vols of 7 M urea, 0.15 M NaCl, 50 mM Tris, pH 7.0, plus protease inhibitors. Bound proteins were eluted in the same buffer containing 1.0 M NaCl, dialysed extensively with buffer containing 10 mM Tris, 10 mM sodium acetate, pH 7.0, lyophilized, and reconstituted with 1/10 vol sterile double distilled water. CaCl_2 was added to 3.0 mg of protein from this preparation to a final concentration of 10 mM, and divided into two aliquots, one of which was digested with 5 units of heparinase II overnight at 25 °C. Undigested and digested samples were purified sequentially on the same affinity column containing 100 µg of affinity purified antibody per ml of Affigel 10 resin. After extensive washing with PBS (100 column volumes) and 1.0 M NaCl (100 column volumes), bound proteins were eluted with acetic acid, pH 2.5, containing 0.15 M NaCl and neutralized by the addition of an equal volume of 1.0 M HEPES, pH 9.0. Eluted proteins were concentrated on Centricon 10 filtration units (Amicon) and iodinated using 1 mCi ^{125}I NaI (16 mCi per µg iodine) with Iodobeads (Pierce Chemical) by the manufacturer's suggested protocols. Iodinated proteins were resolved by electrophoresis on SDS-PAGE on MBS 4217 (ref. 35). Resolving gels were composed of a linear 2.5–20% T; 20% C DATD cross-linked polyacrylamide gradient. Such gels have sufficient porosity to resolve macromolecules of *M*_r approaching 10⁶. Stacking gels contained 2.5% T; 20% C DATD cross-linked polyacrylamide. Gels were fixed, dried and autoradiographed at –70 °C with intensifying screens. Fusion proteins were produced in *E. coli* cells using *PATH* vectors (T. J. Koemar and A. Tzagoloff). A 1.3-kb *Bam*HI–*Hind*III fragment isolated from a *per* complementary DNA (ref. 10) was cloned into a *PATH*3 vector. Induced proteins were prepared by the procedure described by Roth *et al.*³⁶ as described above, and immunoprecipitated by the addition of 1/100 dilution of affinity purified antibody against *per* protein, with or without 1 µg of synthetic peptide, or by 1/10 dilution of rabbit pre-immune sera. Antibody–antigen complex was isolated by adsorption to protein-A–sepharose and resolved by SDS-PAGE³⁵.

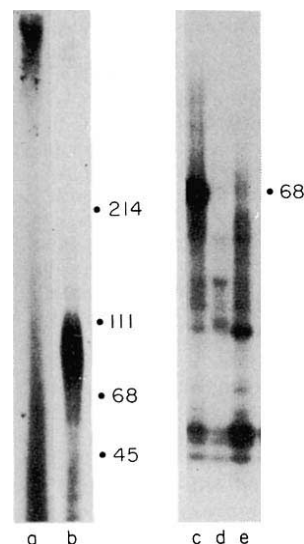
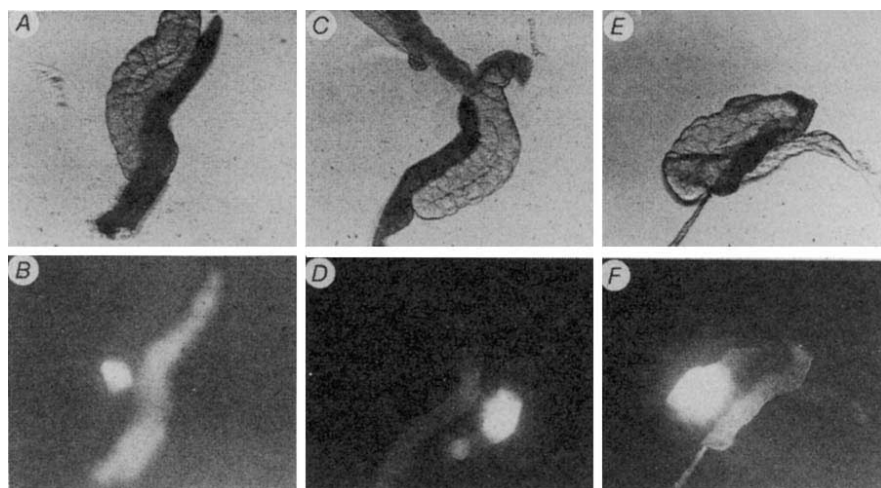


Fig. 4 Dye coupling among salivary gland cells differs in various *per* mutants. Lucifer Yellow CH (5% w/v in 150 mM LiCl) was iontophoretically injected into individual salivary gland cells of *per*⁰ (A, B), wild type (C, D), and *per*^s (E, F) flies. A, C, E, phase contrast micrographs of the injected glands, B, D, F, fluorescence micrographs of the corresponding glands (Olympus inverted microscope with Xenon source, B filter block, 10-s exposure on Kodak Plus X film). Illustrated examples are representative of at least 30 experiments in each group. The *per*⁺ salivary gland illustrated shows two dye injections, with the faint staining on the left being residual dye from an injection 10 min previously. Cell sizes are about 40 μ m (see Fig. 6).



transforming DNA³. Therefore, the differential coupling is not due to the genetic background of *per*⁰.

In contrast to *per*⁰ flies, which display arrhythmic behaviour, *per*^s flies have short-period behavioural rhythms. Cells from salivary glands of *per*^s strains exhibit a more rapid exchange of Lucifer Yellow than wild type, with dye transfer to second and third order cells always detectable within 1 min of injection (Fig. 4). These findings suggest that the extent of dye coupling in the salivary gland is inversely related to the period length measured for *Drosophila* behavioural rhythms. An inverse relationship has also been reported between period length of circadian rhythms and abundance of *per* transcripts in several lines of transformed flies (refs 3, 37 and 38). It has also been suggested that *per*^s mutants produce a hyperactive protein^{3,37,38}.

Strength of electrotonic coupling among salivary gland cells was quantified in several ways, including measurements on intact salivary glands and between cell pairs prepared from isolated glands by mild enzyme digestion (0.1% pancreatin) and hand dissection. The results of these experiments are described in Figs 5 and 6 and summarized in Table 1. With each of the techniques used to quantify spread of electrical current between salivary gland cells, differences among the mutants and between mutant and wild-type glands were striking. Very weak electrical coupling was detected in *y per*⁰, *ry*⁴² salivary glands and *Df(1)TEM202/Df(1)64j4*, *per*⁻ glands (junctional conductance (g_j) values were $0.045 \pm 0.012 \mu$ S ($n = 4$) and $0.21 \pm 0.08 \mu$ S ($n = 7$), respectively, which are not significantly different; data are combined in Table 1). The equivalence of coupling in these

preparations indicates that there is no residual activity in *per*⁰ glands associated with the truncated protein that should be synthesized in these larvae^{3,4}. A more than threefold increase in coupling coefficient and space constant, and a tenfold increase in g_j were evident among cells in wild-type glands (Table 1). Intermediate coupling coefficients (0.3 ± 0.05 , $n = 7$) were observed in the transformed line *y per*⁰, *ry*⁴², P1/25. As expected from the dye permeability studies (Fig. 4), cells from *per*^s salivary glands exhibited substantially greater electrical coupling than wild-type glands (g_j for example, increasing sevenfold: see Table 1). This reinforces the relationship between structural changes in the *per* locus and aberrant intercellular communication.

Junctional conductance (g_j) between wild-type insect salivary gland cells is sensitive to voltages imposed across the junctional membrane, and from the cell or channel interior to the extracellular space^{24,25}. To assess the function of voltage-dependent channel gating in our experiments, we measured resting membrane potentials of 10 *per*^s cells exhibiting strong dye transfer (range -35 mV to -50 mV, mean -42 mV) and 10 *per*⁰ cells characterized by weak dye transfer (range -40 mV to -55 mV, mean -45 mV). The similarity of resting membrane potential in *per* mutants with strikingly different coupling strengths indicates that the differences in intercellular coupling shown in Figs 4-6 and Table 1 are related to alterations in junctional conductance and not to non-junctional membrane-related phenomena. Additionally, we compared the changes in g_j in each of two cell pairs obtained from *per*⁰ and *per*^s in response to a 1-2-min membrane hyperpolarization (by 40 to 70 mV). In all cases, g_j increased

Table 1 Strength of electrical coupling between salivary gland cells of *per* mutants

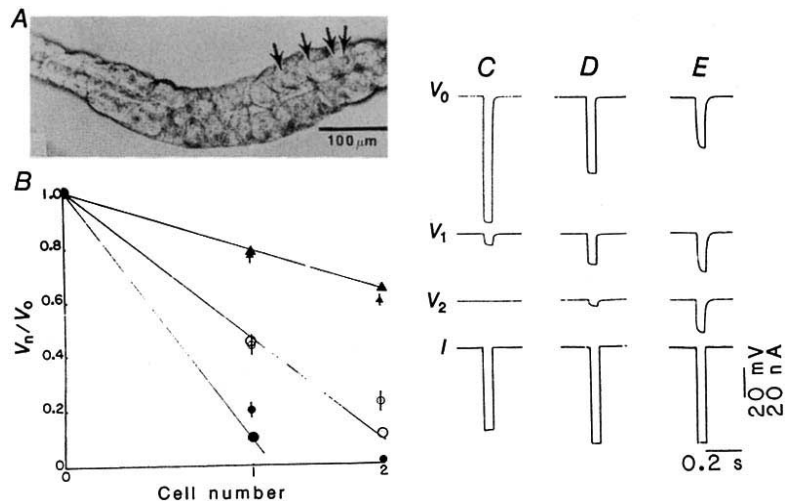
Parameter	<i>per</i> ⁰	Fly genotype <i>per</i> ⁺	<i>per</i> ^s
Coupling coefficient (k)	0.14 ± 0.03 (38)	0.44 ± 0.04 (25)	0.77 ± 0.03 (22)
Apparent space constant (λ')	$32 \pm 15 \mu$ m (19)	$120 \pm 40 \mu$ m (14)	$420 \pm 100 \mu$ m (14)
Junctional conductance (g_j)	$0.17 \pm 0.05 \mu$ S* (11)	$1.57 \pm 0.7 \mu$ S (6)	$10.3 \pm 4.2 \mu$ S (4)

Values are given as mean $\pm$ s.e.m., with the number of determinations, each performed on a separate salivary gland, given in parentheses.

Coupling coefficients were determined with current and voltage electrodes in each of two adjacent cells (1 and 2) in distal cells of salivary glands, in which one or more cells were damaged to reduce nonjunctional current flow. Individual values used for means were averages of values obtained from cell 1 to cell 2 and from cell 2 to cell 1. The apparent space constants were determined in the same way except that separate current and voltage electrodes were placed in one distal gland cell and voltages were recorded in that cell and two others serially adjacent to it. Apparent space constant was obtained for each experiment from plots of electrotonic decay as a function of cell number (ref. 29; see Fig. 5). Cells were assumed to be similar in size (40 μ m) and isopotential in all experiments³⁰. Because the voltage drop is assumed to occur entirely at the junctional region, apparent space constant might be more appropriately expressed as the cell number at which voltage is attenuated by $1 - 1/e$ (=63%). This would be 1, 4 and 10 cells for *per*⁰, *per*⁺, and *per*^s, respectively. Equations used for calculating apparent space constant have been presented elsewhere³⁰. Junctional conductance was determined on dissociated cell pairs with current and voltage electrodes in each cell; g_j values were calculated by application of the pi-tee transform to input, and transfer resistances obtained in response to current pulses passed alternately in the two cells³¹.

* Because values for *per*⁰ ($n = 4$) and *per*⁻ ($n = 7$) were similar, they were combined in calculations of g_j .

Fig. 5 Electrotonic spread within salivary glands differs in *per* mutants. **A**, Arrows, positions for current injection and measurement of electrotonic decay. Two electrodes in cell at farthest right allow current delivery (I) and potential measurement (V_0). One electrode in each of the remaining two cells allows measurement of electrotonic spread (V_1 , V_2). **B**, Voltages in three linearly arrayed cells due to current injected into the first (most right-hand) of the cells illustrated in **A** are plotted semilogarithmically as a function of cell number for representative *per*⁰ (●), wild-type (○), and *per*^s (▲) glands. Sample records from which these values were obtained appear in **C**, **D** and **E** (*per*⁰, wild type, *per*^s, respectively). Each panel is read vertically. Injected current (I) is shown at the bottom of each panel. In **B**, small symbols with error bars represent mean and s.e.m. for experiments from which apparent space constant was determined (see Table 1 for n); large symbols represent data in **C–E**. Values for apparent space constants obtained from a number of such experiments are given in Table 1. Lines connecting the points are for illustration and space constant determination only; each cell is considered isopotential and the resistance of the junctional membrane results in discontinuous voltage drops at the interfaces between the cells which are not shown.



by 30–50%, but in neither case did g_j in *per*⁰ cell pairs approach the conductance measured in *per*^s cells (note that average g_j values for these mutants differ by almost two orders of magnitude, Table 1).

The dye permeability and electrophysiological data are consistent with the interpretation that the coupling described is mediated by gap junction channels (for reviews see ref. 26). Proteoglycans are involved in the synthesis and formation of rat hepatocyte gap junction channels²³. Therefore, it seems plausible that a proteoglycan specified by *per* similarly alters intercellular communication in *Drosophila* by determining the number or organization of functional gap junction channels.

The data presented here provide a possible mechanistic explanation for the wild-type rhythms reported in some cells of explanted *per*⁰ salivary glands¹⁵. It is evident that the *per* gene product is not directly involved in the process which results in circadian changes in membrane potential; instead, it synchronizes the expression of rhythmicity by its function in intercellular communication.

The *per* locus and biological clocks

Our data indicate that the product of the *per* locus modulates intercellular junctional communication in *Drosophila* salivary glands. It is not known whether the *per* product affects gap junction-mediated intercellular communication between cells of other *Drosophila* tissues. If it does, *per* mutants must be able to compensate for the physiological consequences of the altered intercellular communication, or the tissues involved must control non-vital processes, as null mutants of *per* are viable. Note that the *per* locus affects rhythmicity of larval heartbeat. The heart is expected to develop and require junctional communication in late embryonic or early larval stages. In sections of very late embryos, antibodies directed against *per* fusion proteins identify cells that appear to correspond to the developing dorsal vessel (L.S., unpublished observation).

To date, we have been unable to detect *per* transcripts or proteins in the nervous system of *Drosophila* embryos (Fig. 1). However, *per* transcripts have been localized in specific regions of the adult brain (L.S., unpublished data). Evidence from genetic mosaics indicates that this is the tissue focus of the circadian clock phenotype of *per* (ref. 13). It seems likely that the function of *per* in the control of rhythmicity in the nervous system of *Drosophila* is analogous to its function in salivary glands—in the modulation of intercellular junctional communication. This is supported by the observation that the amount of coupling detected in third-instar salivary glands from mutant and transformed larvae is correlated with the period length of

circadian and ultradian behavioural rhythms that map to the adult nervous system³.

We feel it is reasonable to propose that a biological clock in the nervous system might be affected by changes in intercellular junctional communication. Gap junctions constitute the electrical synapse in the nervous system of vertebrates and invertebrates (for review, see ref. 27). Electrical synapses form the basis

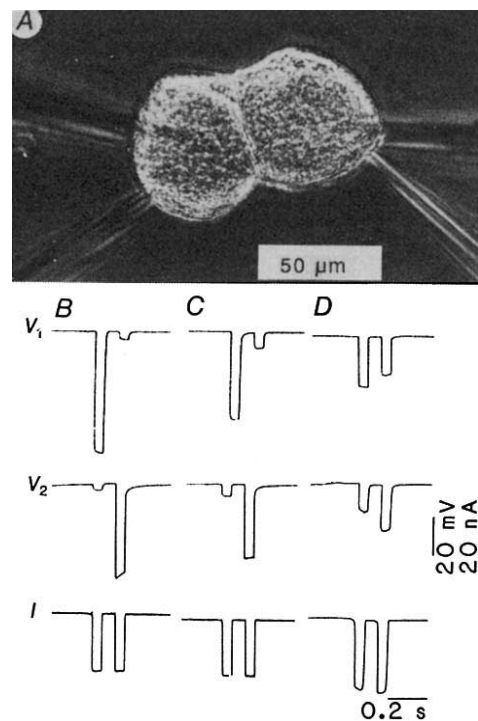


Fig. 6 Electrotonic coupling between dissociated pairs of salivary gland cells differs among *per* mutants. Each of the two cells in **A**, giving voltages V_1 and V_2 respectively, was impaled with separate electrodes (15–25 MΩ, filled with 3 M potassium-citrate or potassium acetate) for passing current (I) and recording voltages. Current pulses passed alternately in the two cells allowed the measurement of input and transfer voltages and the subsequent calculation of junctional conductance (g_j). Values for g_j in *per*⁰ (**B**) wild-type (**C**), and *per*^s (**D**) cell pairs shown here were 0.04, 0.13 and 3.2 μS (see Table 1 for values obtained in various experiments).

for electrotonic conduction. This provides a means for the rapid communication, amplification, and synchronization of signals involved in complex neural and physiological processes²⁶⁻²⁸. Electrical and chemical synapses may interact to form complex systems with a high degree of plasticity²⁶⁻²⁸; gap junction channels are gated electrically and by a variety of second messengers, thereby enhancing the possibilities for variable output from coupled neuronal populations²⁸. A neural system based on the interactions of electrical and chemical synapses, and containing an oscillator, could account for many of the properties of a biological clock. The *per* gene product, by virtue of an effect on intercellular communication, might be central in such a circuit.

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Note added in proof: Extension of the *In situ* hybridization study reported by James *et al.*²⁰ has revealed *per* transcripts in developing salivary glands and CNS (L. Lorenz, J. Hall & M. Robash, personal communication).

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LETTERS TO NATURE

Formation of large-scale structure from cosmic-string loops and cold dark matter

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Cosmic strings are one-dimensional concentrations of energy which can form as the result of a phase transition in the early Universe. Recent interest has been generated by the possibility that strings could lead to the formation of galaxies and large-scale structure¹⁻⁹. Here we present some results from a numerical simulation of the formation of large-scale structure from cosmic-string loops. We find that even though we require $G\mu < 2 \times 10^{-6}$ (where μ is the mass per unit length of the string) to give a low enough autocorrelation amplitude, there is excessive power on smaller scales, so that galaxies would be more dense than observed. The large-scale structure does not include a filamentary or connected appearance and shares with more conventional models based on gaussian perturbations the lack of cluster-cluster correlation at the mean cluster separation scale as well as excessively small bulk velocities on these scales.

Albrecht and Turok¹⁰ have developed a numerical model for the evolution of cosmic strings, and we use the results of their simulation to model the spectrum of strings at late times. In the string model simulated by Albrecht and Turok, strings must be infinite or form closed loops; they cannot have dangling ends. The closed loops act as point masses on scales larger than their

radii⁴, while moving infinite strings can produce wakes in their path⁷. Here we are concerned only with effects of the loops.

We set up a collection of loops and evolve the loop perturbations forward linearly using the numerical simulation of Scherrer¹¹. At late times, the network of infinite strings and closed loops much larger than the horizon forms a collection of brownian random walks. Parent loops break off from this network and enter the horizon with radius

$$F_p = \alpha t_f \quad (1)$$

where t_f is the time at which the parent loop is formed, and $\alpha \approx 2$. The number density of parent loops forming at a time t_f with a radius R_p is given by

$$dn_p = \epsilon R_p^{-4} dR_p \quad (2)$$

where the constant ϵ can be derived from the numerical results of Albrecht and Turok (see ref. 11). Although the network of infinite strings and trans-horizon loops is a collection of brownian walks, we do not expect the parent loops which break off from this network to display any brownian correlations¹¹. The parent loops are therefore placed at random positions inside of a co-moving box.

After a parent loop enters the horizon, it fragments into daughter loops. We take this fragmentation scheme to be a binary process: the parent loop can fragment into two daughter loops, each of which can fragment into two more daughter loops, and so on. This results in a clustering hierarchy for the daughter loops similar to the model of Soneira and Peebles¹², with a two-point correlation function proportional to r^{-2} as observed in the Albrecht-Turok simulations⁸. Each parent loop is assumed to fragment into N daughter loops, where we take $N = 8$; the numerical simulations indicate that $N \approx 5-10$ (N. Turok, personal communication).

The loops induce perturbations in the background matter density; we assume that $\Omega = 1$ (where Ω is the ratio of mass density to critical density in the Universe) and the Universe is

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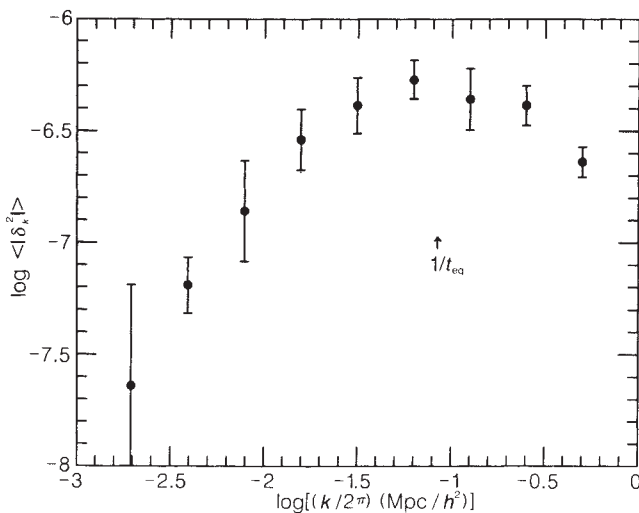


Fig. 1 The power spectrum in the linear regime as a function of the inverse wavelength $k/2\pi$. Error bars give the statistical fluctuations between different realizations. The power spectra are normalized¹⁹ to $1+z=100h^2$, $V=(100 \text{ Mpc}/h^2)^3$ and $G\mu=10^{-6}$.

dominated by cold dark matter. The string perturbations are proportional to the dimensionless parameter $G\mu$, perturbations large enough to produce galaxies are usually assumed to require $G\mu \sim 10^{-6}$. When the strings are formed in a phase transition, a compensating underdensity in radiation is produced in the regions containing the strings¹³, so the total energy density in a region is not altered by the formation of the strings. Thus, the string perturbations have isocurvature, and a given Fourier component can begin to grow only after it enters the horizon. We have approximated this by allowing perturbation growth for a Fourier component only after the co-moving wave length $2\pi/k$ drops below $2t$. The perturbations due to all of the loops which form at a time t_i are Fourier-transformed, and each Fourier component is evolved linearly forward, beginning at the loop formation time or the horizon-crossing time for the Fourier component, whichever occurs later. This process is repeated over a range of values for t_i , and the total Fourier spectrum is inverse transformed to give the final perturbation spectrum at late times.

The linear power spectrum from ref. 11 is shown in Fig. 1. So long as the overall size of the simulation is at least L_{eq} , where L_{eq} is the comoving horizon scale when the Universe becomes matter dominated, and also L_{eq} is not too small to be resolved by the code, we have consistency between various realizations on various length scales. This is due to the importance of perturbations at L_{eq} . All our conclusions are based on runs in which these conditions are met. We find the resulting power spectrum of density perturbations $|\delta|^2 \propto k^n$ has an asymptotic slope $n \sim +1$ for $k \leq k_{eq}$ where $k_{eq} = 2\pi/L_{eq}$, the wavenumber corresponding to the horizon when the Universe becomes matter-dominated, and $n \sim -1$ for $k > k_{eq}$. The bend in the power spectrum at k_{eq} is due to the suppression of the growth of isocurvature perturbations outside the horizon. By testing the distribution of maxima and the three-point correlation of mass we confirm that this distribution is indeed non-gaussian¹¹. The loop perturbations were evolved linearly until the first peak we can resolve becomes nonlinear; then, nonlinear evolution is followed with a standard cloud-in-cell gravitational clustering code, described elsewhere^{14,15}. We simulated a variety of physical scales with $64^3 = 262,144$ particles on an equivalent mesh with several realizations each. We are thus largely free of the effects of discrete behaviour due to the use of a particle code. This is important because we do not want shot noise, which is a source of unphysical perturbations for this dark matter universe. One cross-check with a 128^3 code was made.

We evolve the gravitational clustering code until the correlation amplitude is comparable to that of galaxies, for a Hubble constant $0.5 < h < 1.0$, where h is in units of $100 \text{ km s}^{-1} \text{ Mpc}^{-1}$. (The simulations do not depend on h ; physical scaling of them depends on h^{-2} .) A choice of h is implied by comparison to the autocorrelation of galaxies, where the scale goes as h^{-1} . In Fig. 2a we show a cartesian slice 1-cell thick of one of our simulation cubes $64 \text{ Mpc } (\Omega h^2)^{-1}$ across. The arrows indicate the position and projected velocity of particles found in this slice. Astronomical observers measure distance by Doppler shift, in Fig. 2b we show the apparent positions as seen by an observer at the origin of the plot.

The autocorrelation of the mass $\xi(R)$ (see Fig. 3) has a power-law slope of -2.5 to -3.0 on scales we can easily resolve, which is similar to results found with poissonian initial conditions¹⁶, whereas the observed galaxy correlation slope is about -1.8 . This supports a comment by P. J. E. Peebles (personal communication) that one would expect the region around galaxies to be far more dense in a loop accretion model than is actually observed. The gentle bend in the power spectrum results in a spectrum which resembles poissonian noise over a wide range of length scales.

If we set a length scale by equating r_0 (where $\xi = (r_0/r)^\gamma$) in the simulations to that of galaxies, we constrain $G\mu$, given constraints on Ω and h . An extremely conservative view is $\Omega h < 1$; to fit this we find $G\mu < 6 \times 10^{-6}$. A more widely held view would have $\Omega h < 0.5$, to give sufficient age to the Universe. We find this requires $G\mu < 2 \times 10^{-6}$, in agreement with previous constraints. This improves prospects for this model, because it is independently below limits on $G\mu$ from nucleosynthesis and the cosmic microwave background isotropy¹⁷. Our value is equal to or lower than values usually quoted. This is because we include two additional effects, which are the accretion of matter by loops during the radiation era¹⁸ and nonlinear gravitational growth of perturbations during the late matter era, which is faster than linear.

But this is the only way in which our results support this model. The reduced amplitude of the three point correlation Q is (for scales of 1 to 100 $\text{Mpc } (\Omega h^2)^{-1}$, between 1.5 and 2, somewhat outside the acceptable range of values^{19,20}.

In our runs with $G\mu \sim 2 \times 10^{-6}$, we find that the mean centre-of-mass velocities of spheres of radius $50 h^{-1} \text{ Mpc}$ are 100 km s^{-1} , far below observed values²¹⁻²³, and no improvement over models with gaussian initial conditions²⁴⁻²⁶. It has been suggested²⁷ that an occasional rare large loop, judiciously placed, might account for this. In searching 4,000 independent volumes that are the size of these spheres, we found two with bulk velocities of more than 400 km s^{-1} , and none in the 600 – $1,000 \text{ km s}^{-1}$ range seen in observational data. Raising $G\mu$ helps very little, as it forces a reinterpretation (due to the increased correlation amplitude) of the length scale in the simulations, lowering the velocities on the scales corresponding to the observations and largely cancelling the effect gained by raising $G\mu$. The only way to achieve high velocities here is to assume mass is a great deal more tightly clustered than galaxies, which is equally true in conventional gaussian models^{24,26}. These rather low velocities are a consequence of the suppression of large-scale power resulting from the isocurvature perturbations. In our studies the mass autocorrelations. In our studies the mass autocorrelation goes to zero at about $6 \text{ Mpc } (\Omega h^2)^{-1}$ and is negative beyond. The typical amplitude in this negative region is $-(1 \text{ to } 10) \times 10^{-2}$, many standard deviations below zero. (The high precision is made possible by our thorough suppression of discreteness effects, and is beyond that found in observations.)

It is expected in gaussian fields that the autocorrelation of density maxima goes to zero when the autocorrelation of the field does²⁸. Most popular models for large-scale structures have mass correlations which go to zero at a much smaller radius than that of the observed galaxy cluster-cluster correlations. This apparent conflict is only slightly abated by dynamical

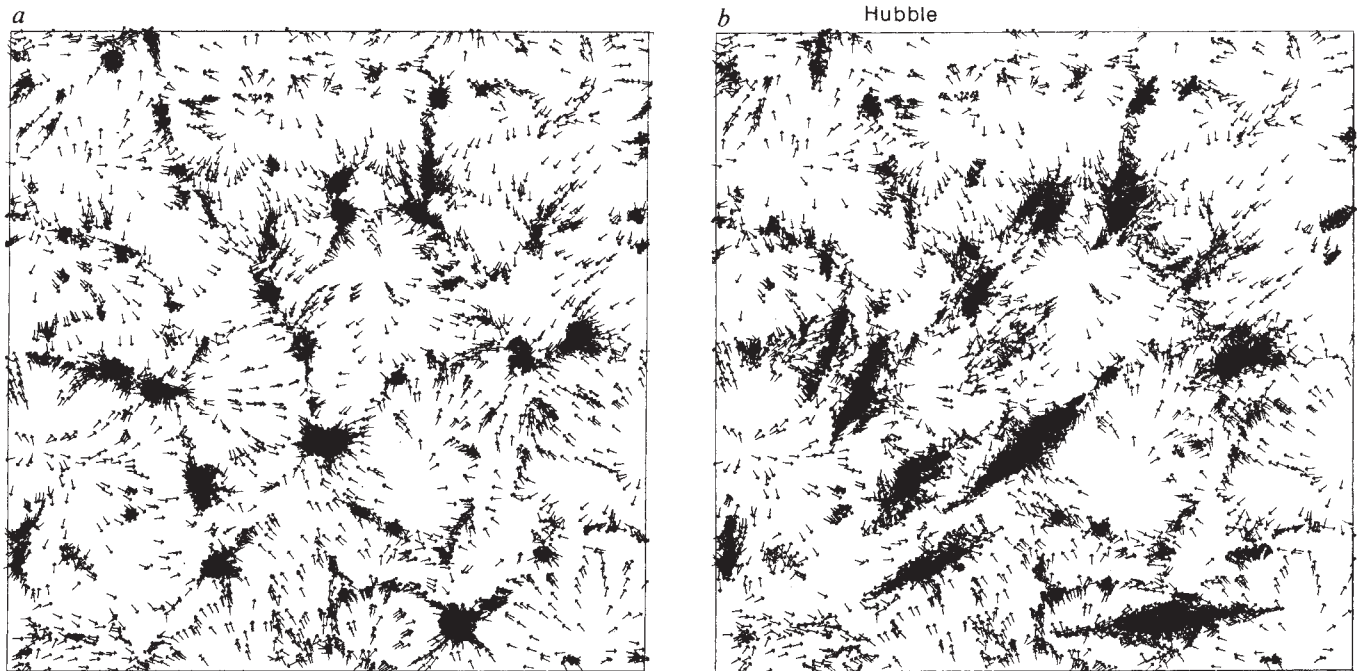


Fig. 2 *a*, A slice $64 \times 64 \times 1 \text{ Mpc } (\Omega h^2)^{-1}$ of a string-loop model. The arrows are centred at the position of simulation particles, and they point in the projected direction of motion. *b*, Same as *a*, except that the position of the particles has been modified to coincide with those assigned by an observer at the origin using redshift as a distance indicator.

evolution of these gaussian models²⁹. Our results show that the hope that this problem could be avoided in string models is so far not justified, either. In Fig. 3 we show the mass autocorrelation in our models as well as a 'cluster-cluster' correlation constructed by identifying density maxima on the cluster mass scale. The cluster-cluster correlation can have enough amplitude at small scales where the amplitude is ≥ 1 , but rapidly drops to zero, in apparent conflict with observations. It is more than 3σ (simulation errors) from the data for $R > 20 \text{ Mpc } (\Omega h^2)^{-1}$, taking $h = 0.5$, and worse for larger h . Of course, the observations can be improved. The correlation of density maxima differs from earlier predictions for the string model of the cluster-cluster correlation function because we have explicitly included the suppression of perturbation growth outside the horizon; it agreed with those expectations before we took this into account. The correlation of maxima depends on all wavenumbers. Suppression of growth of appropriate wavenumbers must be included, and should be present regardless of the details of string evolution. As we include the effect of cluster mergers and other nonlinear processes which occur even when the r.m.s. density contrast is low, our cluster-cluster correlation evolves slightly in the cloud-in-cell code. But even before this evolution the slope is too steep.

This distribution does not look much different from evolved poissonian models, which Fig. 2 also visually resembles. It lacks the frothy connected structures reported by many astronomers in the last decade, although a trace of the feature at k_{eq} is visible. Of course, due to this feature, it has even less power on large scales than a poisson distribution.

The rather poor showing of this model is not very sensitive to many of our interpreting assumptions. (1) Raising $G\mu$ would violate other constraints, for instance, the mass autocorrelation amplitude, and would have very little effect on the zeroes of ξ . (2) Interpreting peaks of density as galaxies, called biasing, makes most fits to data even worse.

Using hot dark matter and strings will improve some things, no doubt. It should provide a frothy appearance, due to the damping of small-scale power. We do not expect the velocities on scales above the damping to be any larger than those studied elsewhere²⁴⁻²⁶, and it is likely that cluster-cluster correlation

will be negative at moderate radii as in gaussian hot models²⁹. It will no doubt lower the correlation amplitude relative to traditional hot models, by removing the necessity to assume galaxies can form only in 'pancakes'.

Models with string wakes^{30,31} hold out some hope of producing better large-scale structure, but these models also have a characteristic scale around L_{eq} . Power on much larger scales is demanded to fit large-scale streaming motions of 700 km s^{-1} and more. Wake-dominated models may (on large scales) resemble models with hot dark matter and strings, but both need to be studied by numerical simulation.

We find that string-loop dominated universes are a poor fit to data on the Universe, and do not see that modifications with wakes or hot dark matter are likely to help with problems on very large scales.

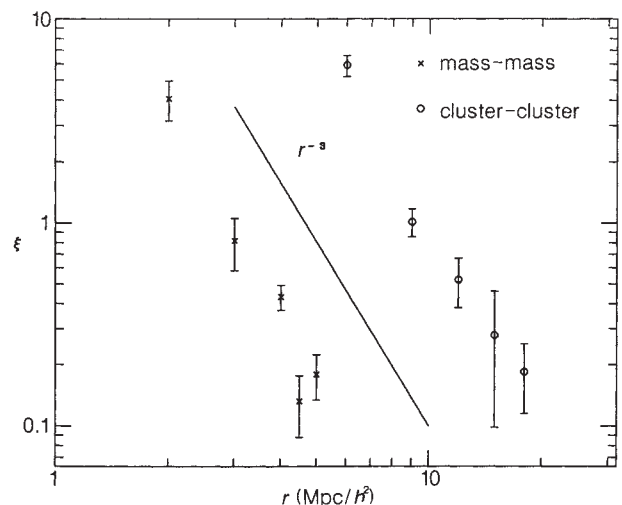


Fig. 3 The left-hand curve is the mass autocorrelation in the simulation, and the right is the autocorrelation of density maxima on a scale of $10^{14} M_{\odot}$, normalized to the same number density as Abell clusters of richness > 0 .

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Note added in proof: Our values for velocities of spheres are in good agreement with a new calculation³² as well as another paper²⁷ we received after submitting this work. These papers do not include the effect of isocurvature suppression on correlations and structural morphology.

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The first images from optical aperture synthesis

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The attainment of diffraction-limited images with large ground-based optical telescopes is an important objective for many astronomical programmes. The limited resolution set by atmospheric fluctuations in refractive index can be overcome by applying aperture synthesis and phase-closure techniques to short-exposure images taken through non-redundant aperture masks. The first optical images obtained with this technique are presented here. The diffraction limit is achieved with good dynamic range even when the number of photons in each short exposure is small ($\ll 100$).

Most previous attempts to obtain high-resolution astronomical images from the ground used short-exposure 'speckle' images obtained by using the whole telescope aperture. Labeyrie's method¹ enables the amplitudes, but not the phases, of the spatial coherence function to be measured and hence does not in general permit unambiguous image reconstruction. Later developments²⁻⁴ have enabled some phase information to be retrieved and true images have been made in a few cases^{5,6}. Our alternative approach is to mask the telescope mirror with an array of apertures each no larger than the scale size r_0 of the atmospheric fluctuations. Short-exposure images can then be analysed to give both amplitudes and phases.

We have already demonstrated⁷ that closure phases can be obtained at high light levels. We have now made systematic observations to measure the spatial-coherence function with this technique at low photon rates and have derived high-resolution images from the data.

Data were taken on 30.10.85 with the Image Photon Counting System (IPCS) on the 2.5-m Isaac Newton Telescope on La Palma. A 136-mm focal length lens behind the $f/15$ Cassegrain focus re-imaged the pupil at a diameter of 9.0 mm. The aperture mask consisted of four holes whose separations gave six uniformly spaced non-redundant baselines (1-6) in one dimension: the hole diameter corresponded to 5.6 cm at the pupil and the

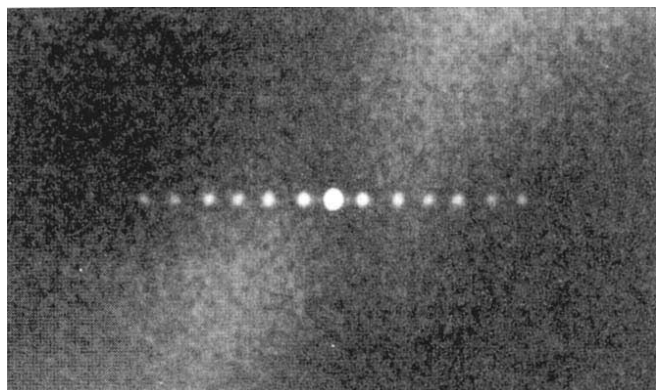


Fig. 1 Computed power spectrum for 7,000 short-exposure images of the calibration star λ Peg.

unit baseline separation to 16 cm. After passing through the aperture mask the collimated beam was refocused onto the IPCS at an image scale of $1.24 \text{ arc s mm}^{-1}$, giving 3.8 pixels per fringe for the finest fringes. An area of $512 \text{ pixels} \times 128 \text{ pixels}$ was scanned every 16 ms and the coordinates of each photon recorded on magnetic tape. A 12-nm (FWHM) interference filter centred on 512 nm defined the bandwidth and no atmospheric dispersion corrector was used. Stellar profiles, observed with the whole of the telescope pupil, had widths of 1.2 arc s, which corresponds to a value of r_0 of approximately 9 cm.

Two of the stars observed were λ Peg, a 3.95-m single star and ϕ And, a binary system with a separation of 0.45 arc s and magnitude difference of 1.2 m. λ Peg is unresolved at a 1-m baseline, providing a good calibration source, whereas the wide double is well resolved at our maximum baseline. Both stars were observed with the aperture mask at nine different position angles separated by 20° : at each position angle ~ 5000 short-exposure images were recorded. Each 16-ms IPCS frame contained an average of only 40 photons, too few for the six sets of fringes to be seen by eye. Estimates of the fringe parameters were made in two stages. Initially, to measure the effective scale of the aperture mask, the summed autocorrelation function of 7000 short-exposure images of the calibration star was computed and Fourier transformed. The resulting centrosymmetric pattern

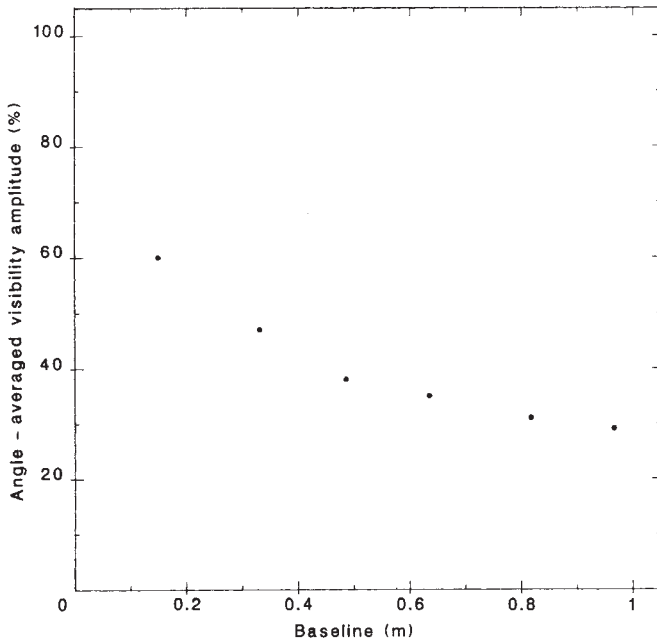


Fig. 2 Observed visibilities of the calibration star averaged over all position angles as a function of baseline.

(Fig. 1) shows six peaks on either side of the origin, each proportional to the squared visibility amplitude of the source at that spatial frequency. The centroids of these peaks define the spatial frequencies of individual baselines. Subsequently, for each separate frame, a discrete Fourier transform was performed at these spatial frequencies to measure the amplitudes and phases of the six fringe patterns present.

It is straightforward to show, by arguments very similar to those in the analyses of both power-spectra⁸ and bispectra⁹, that unbiased estimates of the visibility amplitudes and closure phases can be derived from the series of short-exposure frames recorded at each position angle if allowance is made for photon noise. Only a summary of the results is given here.

If the image, $I_k(x)$, of the k th frame containing N_k photons is represented by a superposition of δ -functions at the positions, x_j , of the photons:

$$I_k(x) = \sum_{j=1}^{N_k} \delta(x - x_j)$$

then the Fourier transform at spatial frequency a , $\tilde{I}_k(a)$, of this image is:

$$\tilde{I}_k(a) = \sum_{j=1}^{N_k} \exp(-i2\pi ax_j)$$

and the unbiased estimate of the normalized visibility for a particular baseline frequency a , is:

$$M \left[\frac{\langle \tilde{I}_k(a) \tilde{I}_k^*(a) - N_k \rangle}{\langle N_k \rangle^2} \right]^{1/2}$$

where M is the number of apertures in the mask and $\langle \rangle$ represent averages over the ensemble of frames.

Unbiased estimates of the closure phase, $\phi(a) + \phi(b) + \phi(c)$, for three baselines a , b and c , forming a closed loop were derived from the quantity:

$$\langle \tilde{I}_k(a) \tilde{I}_k(b) \tilde{I}_k(c) - [\tilde{I}_k(a) \tilde{I}_k^*(a) + \tilde{I}_k(b) \tilde{I}_k^*(b) + \tilde{I}_k(c) \tilde{I}_k^*(c) - 2N_k] \rangle$$

which is identical to the estimate of the bispectrum in speckle

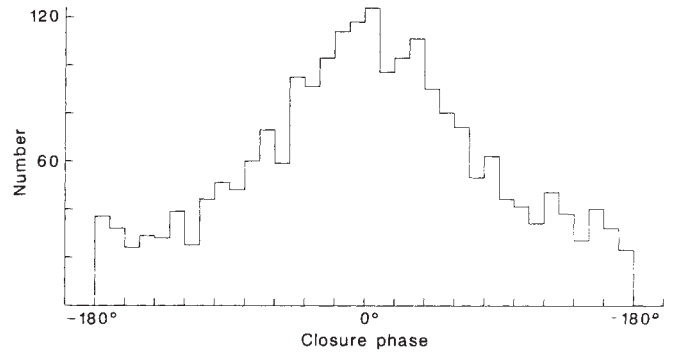


Fig. 3 Histogram of closure phases for 2,500 short-exposure images of λ Peg. Range in closure phase, -180° to $+180^\circ$; maximum count, 124.

masking (ref. 9, (17) and (18)). Its real and imaginary parts are:

$$C = \langle |\tilde{I}_k(a)| |\tilde{I}_k(b)| |\tilde{I}_k(c)| \cos(\phi_k(a) + \phi_k(b) + \phi_k(c)) - [|\tilde{I}_k(a)|^2 + |\tilde{I}_k(b)|^2 + |\tilde{I}_k(c)|^2 - 2N_k] \rangle$$

and

$$S = \langle |\tilde{I}_k(a)| |\tilde{I}_k(b)| |\tilde{I}_k(c)| \sin(\phi_k(a) + \phi_k(b) + \phi_k(c)) \rangle$$

and the unbiased closure phase estimate, $\phi(a) + \phi(b) + \phi(c)$, is $\tan^{-1}(S/C)$.

The observed visibility amplitudes of the reference star show no dependence on mask orientation. The angle-averaged values are plotted against baseline in Fig. 2. The reduction in visibility at the shortest baselines is consistent with that expected due to the presence of random wavefront tilts across each aperture in the mask. The further loss of visibility at larger baselines is due to a number of factors, which include the length of the exposure time, inadequate sampling of the finest fringes, defocus, the finite bandwidth and telescope motion. Although the exact contribution of each of these processes is difficult to establish accurately, it is clear that significant coherence losses are produced by image motion in the focal plane either caused by the atmosphere or by movement of the telescope itself. Hence the design of any future second-generation image-plane interferometer must certainly incorporate a facility to permit independent autoguiding of the separate images formed by each subaperture.

Figure 3 shows a histogram of measured closure phases for 2,500 short exposure images of the reference star. For each position angle, the mean closure phases, computed as described above, were close to the value of 0 degrees expected for an unresolved source and had an r.m.s. uncertainty of about 5° , which is comparable with the typical closure phase errors measured in VLBI. We have compared the observed closure phase distributions with those obtained from numerical simulations of short exposure images containing the same number of photons per exposure. These show almost identical mean values and widths indicating that the large spread in observed values is primarily due to photon noise and not to atmospheric corruption of the recorded fringe patterns. In addition we have found that our algorithm for determining the closure phases is still reliable when as few as five photons are present in each short exposure image. Thus even at relatively low photon rates, accurate estimation of optical closure phases is straightforward and maps of similar quality to those obtained in radioastronomy should be achievable.

A maximum entropy method (D.S. and Gull, in preparation) has been used to reconstruct images of the two stars from the measured visibility amplitudes and closure phases. The width to half maximum of the image of λ Peg (Fig. 4a) sets an upper limit to its angular size of 50 mas. There is no evidence in the image for any departure from circular symmetry except at

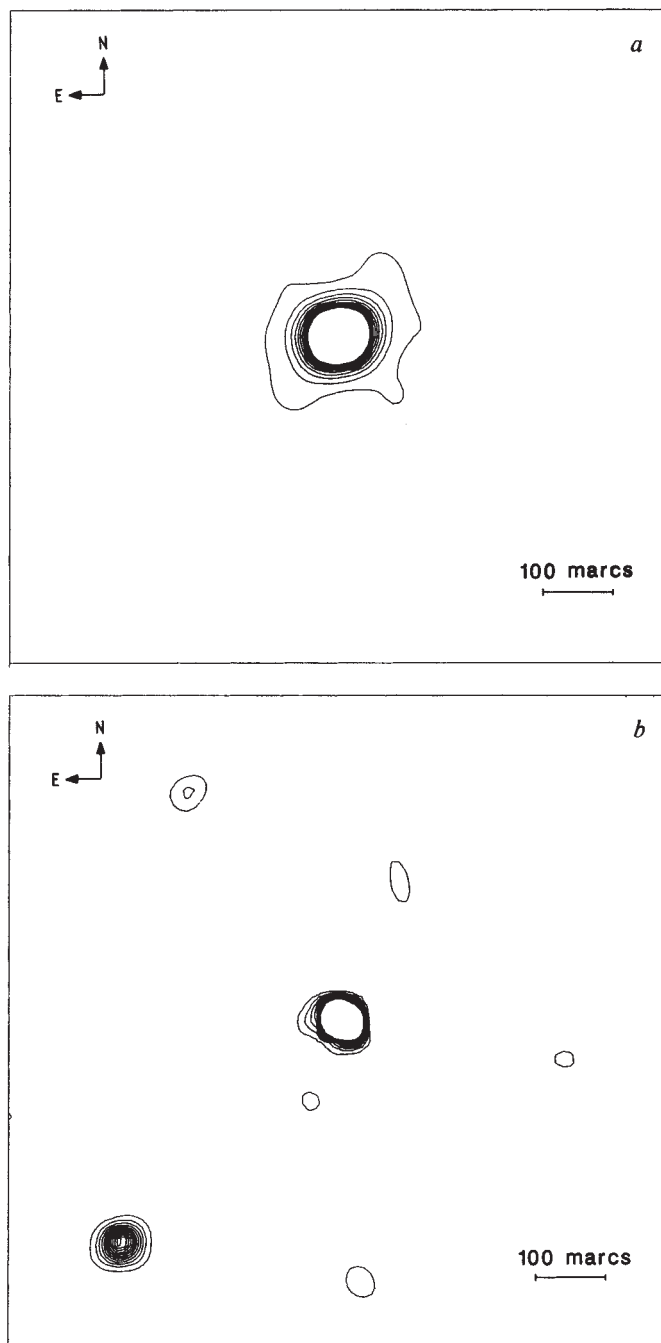


Fig. 4 MEM reconstructions of λ Peg (panel *a*) and ϕ And (panel *b*) contoured at intervals of 1%, from 1% to 15% of the peak intensity.

the lowest contour level (1%), and even at this level few spurious features are present. The effective dynamic range of the image is at least 50:1. The amplitude data as a function of baseline for this star were used to calibrate the observations of the binary ϕ And. The binary star image, (Fig. 4*b*), is again of good quality with very few noise features at the 1% level and a similar dynamic range. As expected, knowledge of the amplitudes and closure phases has permitted a reliable reconstruction without any need of *a priori* information concerning the morphology of the source. Both the separation and position angle of the source can be determined from the map unambiguously. The observed separation and position angle agree extremely well with published values^{10,11}. Our best estimate of the apparent magnitude difference is somewhat larger than the previously accepted value.

The errors quoted correspond to uncertainties in absolute scale and orientation of the aperture mask.

	Separation (arc s)	Position angle	Δm
This observation	0.460 ± 0.005	$135^\circ \pm 1^\circ$	1.5
Published values	0.460	136°	1.2

The images we have produced show that good results can be obtained even at low photon rates. For the simplest image-plane interferometer, such as the one described here, a limiting magnitude as faint as +11 is expected to be achievable if larger apertures and wider bandwidths are used. By working at longer wavelengths, where both the seeing scale size and the atmospheric coherence time increase favourably, and by correcting for image motions in the focal plane, high-quality images should be obtained at much fainter limiting magnitudes.

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Solar torsional oscillations as a signature of giant cells

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Although the existence of giant cells¹ as the fundamental mode of solar convection has long been proposed on theoretical grounds, attempts to detect them observationally have been unsuccessful. During one search, using Mount Wilson magnetograph data, Howard and LaBonte^{2,3} discovered a pattern of latitudinal velocity bands that move from the poles towards the equator in synchrony with the sunspot cycle, and they interpreted this pattern as a torsional wave or 'oscillation' with wavenumber $k=2$ hemisphere⁻¹. Here we suggest that this signal is not in fact an oscillation but represents a modulation of the mean differential rotation caused by a system of giant convective rolls which start at the poles at 11-yr intervals and migrate to the equator in a period of 18–22 yr. Additional evidence for the presence of these rolls is found in the zero offsets in the Mount Wilson data⁴ and in latitude variations of the limb temperature⁵. Thus we argue that the fundamental mode of giant-cell convection in the sun takes the form of equatorward migrating azimuthal rolls. This differs from the 'banana cell' mode suggested by Gilman⁶, and from the poleward propagating rolls reported by Ribes *et al.*⁷.

Simon and Weiss¹ originally suggested that, in addition to granules and supergranules, there should be a third scale of convection which they called the 'giant cells', having transverse dimensions of $\sim 300,000$ km, and extending over a significant fraction of the convection zone. Gilman⁶ has developed a detailed model of solar convection in which the largest structures take the form of 'banana cells' aligned with the rotation axis. But attempts to detect them have so far been unsuccessful⁸ and it may be that the Sun is operating in an entirely different mode. Thus it is of some importance that other models be considered.

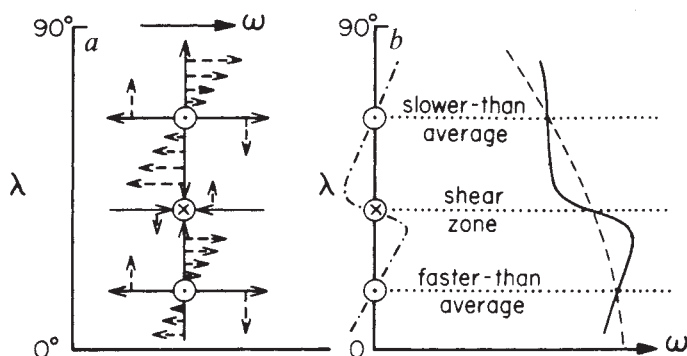


Fig. 1 *a*, The sketch shows qualitatively the Coriolis accelerations (broken arrows) corresponding to the transverse motions (solid arrows) at latitudes of upflow and downflow. *b*, Shows qualitatively the modulation of the mean differential rotation by the Coriolis accelerations arising from the meridional motions of the giant convective rolls postulated in Fig. 2*b*. $\odot$, Upflow; $\otimes$, downflow; ----, mean differential rotation; - - - -, giant cell modulation; —, actual angular velocity.

It was during one attempt to find the giant cells that Howard and LaBonte^{2,3} discovered the so-called torsional oscillations. These take the form of alternate latitude bands of faster-than-average and slower-than-average rotation (as compared with the mean differential rotation for that latitude), which are initiated near the poles at 11-yr intervals and migrate to the equator over a period of 18–22 yr. Howard and LaBonte noted that, as the faster band passes into sunspot latitudes, its poleward shear boundary coincides with the centre-of-gravity of active region formation during the conventional 11-yr cycle.

We have independently criticized the 'wave' interpretation^{9,10}. Snodgrass⁹ has obtained a modified net pattern using a simpler analysis and has investigated the torsional shear¹¹, that is, the derivative of the torsional pattern with respect to latitude. The shear pattern bears a close relationship to the butterfly diagram, although it begins several years earlier at higher latitudes, and it can be argued that this and other activity phenomena should be regarded as part of an extended 18–22-yr activity cycle (P.R.W., R. D. Altrick, K. L. Harvey, S. F. Martin and H. B. Snodgrass *et al.*, in preparation).

Wilson¹⁰ has noted that significant radial displacements of plasma, dr , should result in changes, $d\omega$, in the angular velocity, ω , according to the relation

$$d\omega/\omega = -2 dr/r \quad (1)$$

except along the polar axis, which is a singularity. For meridional motion through a change in the colatitude $d\theta$, the corresponding result is

$$d\omega/\omega = -2 d\theta \cot \theta \quad (2)$$

although Reynolds stresses will tend to reduce the amplitude of these changes.

It must be noted that the angular momentum effect, as defined here, is only one of several dynamical effects at work in the convection zone. Considered in isolation it would yield a rotation rate that increases with latitude, contrary to what is observed, but Gilman¹² has shown that the sign of the differential rotation signal arises as the net result of the Coriolis accelerations acting on all components of the convective flows. Elements moving horizontally in the direction of rotation, that is, faster-than-average rotating elements, are accelerated towards the equator, while slower-than-average elements are accelerated towards the poles. The net sum of all these effects, acting on cells of all sizes, must yield the observed differential rotation.

Individual cells, however, should produce a modulation of the mean differential rotation through the angular momentum effect, and it is suggested that, in searching for evidence of

large-scale convective motions, this 'angular momentum' effect might provide an important clue. Ribes¹³ has also recognized its importance. For granule (lifetimes ~ 10 min) and even supergranule (~ 1 day) size cells, the modulation would be lost in the averaging processes required to resolve the large-scale pattern. But if giant cells with dimensions comparable to the depth of the convection zone and lifetimes of the order of months or more do exist, the modulation of the differential rotation should be observable and may provide a clue to the configuration of these cells. Indeed, if no significant time-dependent modulation of the differential angular velocity could be found, one could reasonably argue that organized convective motions larger than some threshold size, for example, that of the supergranule cells, do not exist.

There is, however, ample evidence^{14,15} of large-scale time-dependent anomalies or variations in the previously assumed uniform differential rotation of the Sun, and thus the nature of the modulations which might be caused by large-scale cells is worth examining. Strictly radial upflows should reduce the mean angular velocity of the local region, following equation (1). More significantly, equation (2) shows that poleward meridional flows ($d\theta < 0$) initiated at a region of upflow should produce an increase in angular velocity (or spin-up) locally proportional to $d\theta$, and that this effect should increase at higher latitudes. Conversely, equatorward flow should similarly reduce the angular velocity in proportion to $d\theta$. This effect is illustrated diagrammatically in Fig. 1*a*. Further, if regions of upflow, indicated by $\odot$, and downflow, $\otimes$, are localized at different latitudes, a velocity shear zone would develop at or near a latitude of significant downflow, and this is also shown in Fig. 1*a*.

When these velocities are superimposed qualitatively on the mean differential rotation, as in Fig. 1*b*, zones of slower rotation, faster rotation and shear are readily reproduced and the resemblance to the torsional oscillation signal is striking. We therefore propose that this signal is a modulation of the differential rotation produced by the Coriolis accelerations arising from giant cell convection.

The cells take the form of a system of large-scale latitude and time-dependent azimuthal convective rolls, occupying (approximately) the lower half of the convection zone. Above this region the density stratification will transfer the main convective flux to smaller eddies, but we suggest that this surface modulation of the differential rotation provides a signature of the presence of the giant rolls. By postulating that they begin at the poles as annular toroids at 11-yr intervals and propagate to the equator in 18–22 yr, the torsional oscillation pattern may be qualitatively reproduced. At lower latitudes, the rolls may break up into a system of giant cells, so that the pattern exhibits some longitudinal structure, but it is assumed that the regions of upflow and downflow continue to correspond to discrete latitudes during this progress.

In Fig. 2, a diagrammatic representation of the predicted pattern is given at four phases of cycle 20, the latitudes of upflow and downflow being chosen consistent with the progression of the torsional oscillation signal. The model does not, of course, provide a physical theory of the convection zone but it does provide a plausible picture, suggesting the direction in which a physical model may develop as a result of further observation and analysis. In particular, it permits predictions about the expected latitudes of the subsurface regions of upflow and downflow, and about the directions of the subsurface meridional flows at different phases of the cycle (shown by the arrows at the peripheries of the diagrams in Fig. 2). Although one would not expect that these flows would be readily observable in the surface velocity fields, they may be detectable by tracer or statistical techniques and can be searched for during the coming cycle.

Two useful lines of testing are suggested by recent and quite different sets of observations. Snodgrass⁴ has examined the relative latitudinal "zero offsets" of the Mount Wilson velocity

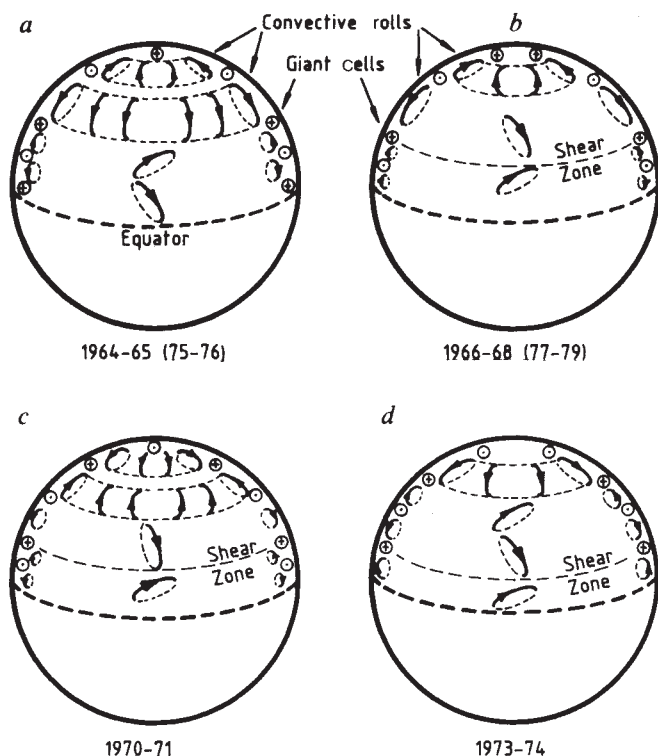


Fig. 2 Sections of the streamlines of a postulated giant convective roll and cell pattern at four phases of cycle 20 are shown. Latitude zones of upflow and downflow are represented by $\odot$ and $\oplus$ respectively, while the expected meridional flows are indicated by the arrows at the perimeter of each diagram.

data, which are the companion data to those from which the torsional oscillations are obtained. From these he has deduced the latitude-by-latitude variations in the line of sight components of the velocity fields which, averaged over 273-day periods, are shown as a contour plot in Fig. 3. The solid lines in this figure represent motion away from the observer and the broken lines towards the observer. The inner contours represent velocities of 4 m s^{-1} .

For large-scale flows in the surface layers of a compressible plasma, continuity requires that the ratio of the radial (or vertical) to the transverse velocities is $\sim H/L$, where L is the transverse dimension of the flow and H the local scale-height. Since, for flows on the scale of giant cells, this ratio is of the order of 10^{-3} , one would expect that the meridional components of such cells would provide the major contribution to the line-of-sight contours, except possibly near the equator. Thus the downflows of such cells may be inferred near the poleward boundary of the solid line contours (indicating convergence) and upflows at the corresponding interface of the broken-line contours.

It is of interest to test the regions of upflow and downflow of Fig. 2 by mapping them on to Fig. 3. Approximately 60% of these points satisfy this criterion, which is encouraging, but no strong confirmation can be claimed. The azimuthal roll model shown in Fig. 2 does not allow for local variations in the velocity field pattern (that is over a period of less than a year) or for differences between the hemispheres, both of which are evident in the Mount Wilson velocity patterns, and this may explain some of the discrepancies. However, a puzzling feature of the data is the unexpectedly large values of the line-of-sight velocities near the equator, where, one would expect, the radial velocity should provide the major contribution to the line-of-sight component, and an investigation of this is being undertaken. Meanwhile, these data should be treated with some caution, but similar observations should be attempted during the coming cycle.

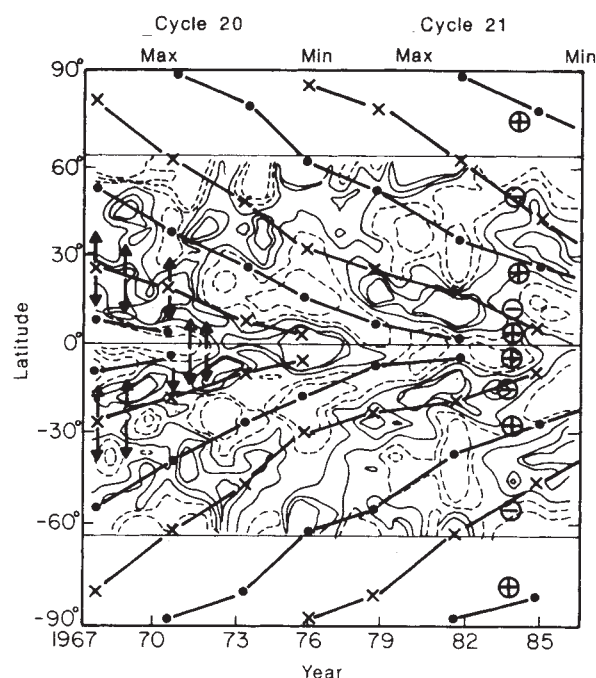


Fig. 3 The synoptic chart shows contours of line-of-sight velocity residuals towards (broken lines) and away (solid lines) from the observer. These are compared with zones of upflow (---) and downflow (-.-) derived directly from Fig. 2. Also shown are points $\oplus$ and $\ominus$ representing temperature excesses and deficits measured by Kuhn *et al.*⁵ in 1983 and the diverging arrows obtained from Ribes¹⁴.

A second and very different kind of test is suggested by the work of Kuhn *et al.*⁵, who have recently measured variations in the limb brightness temperature as a function of latitude during 1983. They find a temperature deficit of order 0.8° near latitude 53° and excesses and deficits of comparable magnitudes at other latitudes (see their Fig. 5). These have been plotted on the synoptic chart in Fig. 3 and the agreement with the latitudes of upflow and downflow interpolated from Fig. 2 is striking, suggesting that the azimuthal rolls postulated here in order to explain the torsional oscillation signal may indeed be convective in origin.

Kuhn warns that the low latitude points may be unreliable due to facular contamination of the signal, but claims that the deficit at 53° is significant (J. R. Kuhn, personal communication). In a later study¹⁶ Kuhn *et al.* consider the possibility that this deficit does indeed correspond to a large-scale convective pattern, and argue that, because the shift in their distribution functions with latitude is significantly larger than their half widths, whatever is responsible for the temperature deficit near 53° has a greater latitudinal than azimuthal structure. This supports our contention that, at these latitudes, the large-scale convective motions take the form of rolls rather than azimuthally structured cells.

However, if this interpretation of the deficit is correct, our model predicts that it should progress to lower latitudes during the cycle at a rate of order $\sim 4^\circ \text{ yr}^{-1}$ (the rate at which the torsional oscillations and the roll pattern move from pole to equator), although the predicted breakup of the roll into giant cells at lower latitudes might compound the observational difficulties. Thus it is of some interest that the most recent observations of Kuhn *et al.*¹⁶ show this minimum at $\sim 49.5^\circ$ in 1983 (due to recalibration), $\sim 47^\circ$ in 1984 and $\sim 45^\circ$ in 1985. While they describe this as, at most, "a weak cycle dependence", the observed shift is in the equatorward direction, although at only about half the rate predicted by the model described here.

Again, while no firm conclusions can be drawn at this stage, further observations during the coming cycle should be of considerable value.

Also shown on Fig. 3 are diverging arrows plotted on a similar synoptic chart by Ribes¹⁷ between 1967 and 1973, and deduced from the motions of young sunspots. Ribes and coworkers^{7,17} claim that these surface motions indicate the presence of convective rolls which are generated near the equator every two or three years and propagate toward the poles, arguing from the poleward trajectories of H_α filaments, which she also shows. Later, referring to a localized (non-global) description of the torsional oscillation signal, Ribes argues that this signal arises from a 'flection' in the differential rotation profile due to a system of poleward propagating rolls¹³.

Snodgrass⁹, however, has shown that the torsional oscillations are more accurately described by a net pattern, propagating from pole to equator, which is consistent with the original description by LaBonte and Howard^{2,3}, and it is hard to see how the interaction between a poleward propagating system of rolls and a stationary differential rotation profile could produce the observed equatorward-propagating net torsional pattern. Thus Ribes' system of poleward-propagating rolls is not the same as that described here and is not consistent with the torsional oscillation signal. Further, the apparent equatorward drift of the limb temperature deficit¹⁶ would, if it proves to be real, suggest that, whatever they may be, the phenomena reported by Ribes are not a fundamental convective mode.

In this context, however, it should be noted that the velocity contours of Fig. 3 do show occasional branches extending towards the poles (for example, at latitude 20–30° between

1982–84) and similar evidence of poleward propagation is also found in the net torsional pattern¹¹, the H_α filaments and the decaying active-region fields. This problem is not addressed by the simple model described here but is discussed by Snodgrass¹¹. In a later paper we will argue that the fundamental convective roll pattern is associated with a dynamo wave and does indeed propagate towards the equator but may give rise to a poleward propagation at higher levels, either as a reflection phenomenon or as the result of a bifurcation of a more basic cell such as the banana cell suggested by Gilman⁶. Further study and observations are obviously needed and are being undertaken during the Solar Cycle Workshop.

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Characteristics and mass distribution of extraterrestrial dust from the Greenland ice cap

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Extraterrestrial grains with sizes $\geq 50 \mu\text{m}$ can now be extracted in large numbers from terrestrial sediments but their origins are still uncertain. At least 99% of these grains have been thought to be spherules^{1,2} resulting from the melting of their parent bodies in the atmosphere, which destroyed their mineralogical composition. Here we present analyses of extraterrestrial grains extracted from six samples of cryoconite³ (black dust) collected from the melt zone of the Greenland ice cap. In addition to families of grains never reported before, we have found a surprisingly high abundance of unmelted chondritic fragments, in which the non-volatile component of the parent bodies has been well preserved. The mass distribution of the grains is very similar to that of the micrometeorite flux at 1 AU (ref. 4), indicating that most of the grains are micrometeorites, and not ablation products of larger meteorites. These results yield new constraints on the ablation of such Greenland micrometeorites, as well as guidelines for using them as glaciological and geological markers.

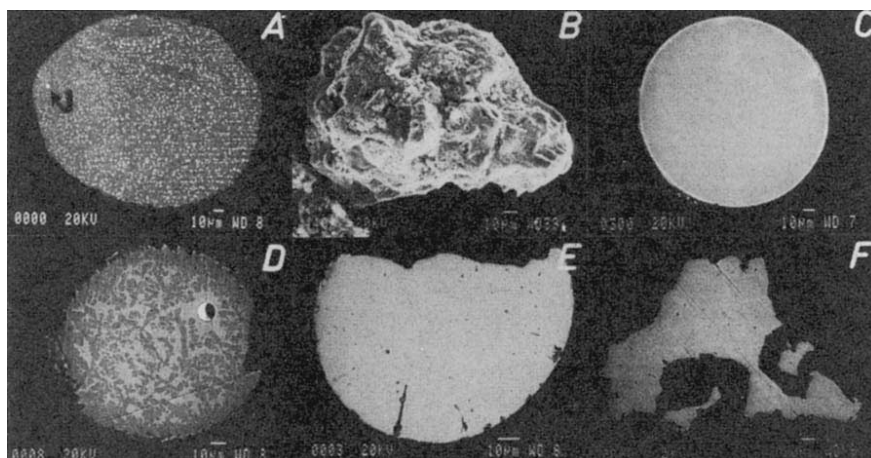
In a preliminary analysis⁵ of extraterrestrial grains extracted from a few kilograms of cryoconite (sample SON-1), we concluded that massive deposits of cryoconite found on the Greenland ice cap constitute the richest and best preserved mines of cosmic dust grains found on the Earth so far. However in this

preliminary sample, still heavily contaminated with terrestrial grains, we reported a very small abundance ($<1\%$ of the total number of cosmic dust grains) of the most interesting family of 'unmelted' extraterrestrial fragments (see below).

We have now analysed grains from five additional samples of cryoconite. Two samples were collected during the same expedition near the same site, at the latitude of Sondrestromfjord, from both a surface pile of sediments (sample SON-2) and cryoconite holes (sample SON-3) that constitute an essential part of the melt zone surface. Three samples were returned from the Jakobson ice field (about 500 km to the north of Sondrestromfjord): samples JAK-1 and JAK-2 were collected at ~ 10 km from the ice margin by J. M. Gauthier, on the bottom of a nearby blue lake and the bank of a torrent, respectively; sample JAK-3 was collected on the bank of another torrent, ~ 25 km further inland on the same ice field, by W. Harrison (University of Alaska) and K. Echelmeyer (Caltech).

All grains were first preselected with the X-ray spectrometer of a scanning electron microscope, by relying on simple criteria previously used for deep-sea spherules¹. We next determined their individual Ir (see Table 1, column 3), Ni, Co and Cr concentration by INAA, for further supporting their extraterrestrial origin⁶. Sample JAK-3, that was found to be highly concentrated in extraterrestrial dust, greatly helped in defining the major families of extraterrestrial grains listed below, including new families either not observed in deep-sea collections¹ or not quoted appropriately in our earlier investigations^{2,5} (see also Table 1 and Fig. 1 for further details about the grains and/or comparison with deep-sea spherules, respectively). These families, presented in descending order of abundance, include: (1) chondritic-stony spherules (Fig. 1A); (2) 'unmelted'-chondritic fragments (Fig. 1B), which are much more abundant than previously thought; (3) new chondritic glassy spherules (Fig. 1C); (4) oxidized Fe/Ni spherules made of wüstite and magnetite (see dark and bright phase in Fig. 2D, respectively), which are relatively rare; (4) new metallic (non-oxidized) Fe/Ni grains including spherules (Fig. 1E) and unmelted fragments (Fig. 1F).

Fig. 1 Scanning electron microscope (SEM) mosaic of the major families of Greenland micrometeorites (their relative abundance, in terms of number of grains, has been listed in Table 1, column 2). **A**, Chondritic-stony spherules: this most abundant type of magnetic particle is also found in deep-sea sediments where they have been much more heavily weathered. They are formed with barred olivine and thin lamellae of interstitial glass with abundant bright crystals of magnetite. The brightest inclusion identified by the dark arrow is a new type of platinum metal nugget in which all the iridium is concentrated. **B**, Unmelted-chondritic fragments: this type of abundant particles with irregular habits and measurable contents of S, are extremely rare in the deep-sea collections (generally obtained by magnetic raking) as most of them are weakly magnetic and/or easily weathered. **C**, Chondritic-glassy spherules: these glasses have not been found in deep-sea sediments. They belong to two subgroups: (i) the 'homogeneous' glasses (**C**) show no structure even at the highest magnification in the high-contrast mode of the SEM; moreover they are not magnetic and strongly depleted in iridium; (ii) the 'barred' ones, that are weakly magnetic, show an ultrafine barred texture of amorphous material and a low density of very small magnetite inclusions. They look somewhat intermediate between the chondritic-stony spherules and the homogeneous glass spherules. They contain iridium concentrations comparable to the value measured in the chondritic-stony spherules. **D**, Oxidized Fe/Ni spherules: these relatively rare spheres have also been observed over the past ~100 yr in deep-sea sediments¹⁸. **E**, A new family of non-oxidized Fe/Ni spherules, as yet only observed in the Greenland sediments. **F**, Unmelted-unoxidized Fe/Ni particles: these very rare grains (less than 2% of the unmelted fragments) have not been observed in deep-sea sediments.



The chondritic composition of the stony and glassy particles, the Ni content of the Fe/Ni grains, and the high Ir concentrations generally measured in all grains (see Table 1, column 3), support their extraterrestrial origin. But the nature of their parent bodies (either interplanetary dust particles or much larger bodies such as meteorites) cannot be inferred from these analyses. We attempted to tackle this problem, starting with the similar size distributions (see line 1 to 4 in Table 2) that we measured for the four most abundant families of Greenland cosmic dust grains. This similarity is clearly illustrated in Fig. 2 where the two top histograms represent the size-frequency distributions of the chondritic-stony spheres and oxidized Fe/Ni spheres, respectively, that are very distinct from those reported for a variety of natural dust grains, such as those found for example in regoliths^{7,8}, resulting from a process of comminution-agglutination associated with repeated impact cratering. This suggests that the corresponding mass-frequency distribution of these grains might bear some unique information about their origin.

This distribution is first generated by transforming the accurate size distribution measured for ~2,000 chondritic-stony spherules (upper left histogram in Fig. 2) into a mass distribution, and taking into consideration the relative mass abundance of the other families of extraterrestrial dust. The resulting distribution,

determined for grains in the 50–300 μm size range, was expressed in units of mg of extraterrestrial component per gram of wet sediment (Table 2, line 6). We next extend it to smaller grains of the <50 μm -residue, by measuring the total mass of iridium in magnetic separates from both this residue ($\sim 1.75 \times 10^{-9}$ g per kg of sediments) and the >50 μm -size fraction ($\sim 2.5 \times 10^{-5}$ g per kg), and normalizing the grains in the <50 μm residue to the >50 μm size fraction.

A conversion to flux units was further required for direct comparison with the micrometeorite flux⁴ in the interplanetary medium at 1 AU, expressed in units of g of extraterrestrial material per m^2 of collector area per year of exposure in space ($\text{g m}^{-2} \text{y}^{-1}$). From our field studies at the Sondrestromfjord site, we found that the concentration of extraterrestrial dust ($\sim 4,000$ grains with a size of >50 μm per kg of sediment) as well as its size distribution, are already established in the thin deposits observed on the bottom of the countless ~10 cm-deep cryoconite holes that constitute an essential part of the melt zone surface. The amount of cryoconite thus deposited per m^2 of the ice surface ranges somewhat between 0.2 kg and 2 kg. By taking a value of 2 kg, that corresponds to fields of the most 'mature' cryoconite holes, which have been stable (and thus effective as cosmic dust collectors) over the longest period, we deduce that

Table 1 Characteristics of Greenland micrometeorites

Type	Abundance	Ir content	Aspect	Magnetism
Chondritic grains				
Stony spheres	50%	10–3,000 p.p.b.	tarnished grey	strong
Unmelted fragments*	25–30%	10–1,000 p.p.b.	tarnished grey	weak
Glass (homog.)†	10%	<10–70 p.p.b.	transparent	no
Glass (barred)†	10%	<10–450 p.p.b.	brownish	weak
Fe/Ni grains				
Oxidized spheres	2%‡	3–20 p.p.m.	dark/shiny	very strong
Metallic spheres	<0.5%	5–8 p.p.m.	yellow/shiny	very strong
Metallic fragments	<0.5%	6 p.p.m.§	yellow/shiny	very strong

* These grains seem to be closely related to CI chondrites with regard to their major element composition, as displayed on Si–Mg–Fe ternary diagrams. But both their petrological features and trace element contents already indicate that they are much more diverse than this peculiar class of primitive meteorites¹⁹.

† See caption Fig. 1.

‡ This abundance is much smaller than the value of ~50% generally quoted for the deep-sea collections¹, but it is rather similar to the corrected value reported by Kyte²⁰, who took into consideration the preferential loss of deep-sea chondritic-stony spherules by weathering.

§ These grains contain the high concentrations of Ga (~14 p.p.m.) and Ge (~260 p.p.m.), expected for extraterrestrial Fe/Ni alloys²¹.

Table 2 Size and mass distribution of Greenland micrometeorites

Type	Size fraction (μm)					
	<50	50–100	100–150	150–200	200–250	250–300
Size distribution						
Stony spheres	—	1,500	300	120	60	20
Unmelted fragments	—	780	265	—	45	—
Glass	—	200*	85	40	15	4
Oxidized Fe/Ni	—	80	20	5	2	—
Mass and flux						
Iridium (10^{-9} g kg^{-1})	1.75	2.5	—	—	—	—
Mass distribution (mg g^{-1})	2.6	3.75	2.0	1.6	1.2	1.0
Flux ($10^{-6}\text{ g m}^{-2}\text{ yr}^{-1}$)	1.75	2.5	1.35	1.05	0.8	0.60

* Lower limit, based on spherule counts only. We did not attempt to separate extraterrestrial glass 'fragments' from a very important terrestrial background of transparent crystalline grains.

this amount of sediment is equivalent to a collector surface of 1 m^2 , exposed for a duration, ΔT , in the cosmic dust flux.

The value of ΔT ($\sim 3,000\text{ yr}$) was inferred from an ice flow model of Reeh⁹, specifically developed for estimating the age of the ice surface at the Sondrestromfjord site, $\sim 2,000\text{ yr}$. The higher value of ΔT accounts for a double source of cosmic dust grains feeding cryoconite holes: about two thirds of the grains are released upon the melting of the successive annual ice layers in which they were first deposited further inland, and that subsequently intersected the melt zone surface during their outward motion over the past $\sim 2,000\text{ yr}$; the remaining one third results from the direct capture of the cosmic dust infall by the most mature cryoconite holes (now found on the $\sim 2,000\text{ yr}$ old surface at the Sondrestromfjord site) that moved at a higher speed on the top surface of the ice cap over the past $1,000\text{ yr}$.

Thus, at the Sondrestromfjord site, each kg of cryoconite corresponds to a collector surface of $\sim 0.5\text{ m}^2$, exposed for $\sim 3,000\text{ yr}$ to the recent extraterrestrial dust infall. This equivalence allows the right conversion of units ($\text{g m}^{-2}\text{ yr}^{-1}$),

and the flux of extraterrestrial dust (lower histogram in Fig. 2) appears strikingly similar (at least with regard to its shape which is independent of our modelling exercises) to the micrometeorite flux at 1 AU (second histogram from bottom). In this comparison we assumed that micrometeorite flux was constant over the past $\sim 2,000\text{ yr}$, and for the convenience of presentation the mass scale of Grün *et al.* was 'reconverted' into a size scale, using a density of chondritic particles of $\sim 2.5\text{ g cm}^{-3}$, already used by these authors for establishing a relationship between size and mass. This indicates that most extraterrestrial grains down to small sizes, are indeed micrometeorites and not ablation products of much larger meteorites. We thus can generalize the important conclusion of Raisbeck *et al.*^{10,11} deduced from individual ^{10}Be and ^{26}Al measurements over a much broader range in both deep-sea and Greenland spherules with sizes larger than $\sim 400\text{ }\mu\text{m}$. As most micrometeorites down to mass $\sim 10^{-6}\text{ g}$ have been shown to follow cometary orbits^{12,13}, this, along with petrographical observations and elemental abundances, suggests that unmelted chondritic fragments with sizes $>100\text{ }\mu\text{m}$ (mass $>10^{-6}\text{ g}$) might represent the non-volatile fraction of dark cometary 'sands', responsible for the very low albedo of cometary nuclei¹⁴. Such 'Greenland cryoconite' micrometeorites (GCM) are much more massive than interplanetary dust particles currently obtainable from stratospheric collections¹⁵, which might also originate from comets.

Some of our observations represent new 'ablation constraints' on the nature of micrometeorites. The similar size distributions of grains that are as different as chondritic and Fe/Ni spheres, suggest that their parent bodies suffered a similar extent of ablation. Moreover the high proportion of 'unmelted' chondritic grains along with the shape of the GCM mass distribution might reflect the occurrence of a very efficient cooling mechanism, that shielded large grains (size $>100\text{ }\mu\text{m}$) against melting and fragmentation. These two last constraints further suggest that the parent bodies of these grains are not likely to be chunks of compact solids such as meteorites, for which reliable computations¹⁶ predict a strong decrease in the survival probabilities upon ablation in the atmosphere for projectile sizes in excess of $\sim 100\text{ }\mu\text{m}$. This should have produced some marked discontinuity in the GCM mass distribution around sizes of $\sim 100\text{ }\mu\text{m}$, which is not observed in Fig. 2.

In Greenland the number of extraterrestrial grains per m^2 of fields of mature cryoconite holes could indicate the melting history during the ice flow. Moreover, the ratio of extraterrestrial to terrestrial matter in these holes will certainly help in probing the circulation history of local aerosols on the ice cap. In much older deep-sea sediments the ratio of the concentration of Fe/Ni cosmic spherules (with sizes $>50\text{ }\mu\text{m}$) to our estimate of the flux of such spheres in Greenland (90 m^{-2} per $1,000\text{ yr}$), should yield sedimentation rates that are very difficult to determine from stratigraphic data alone in specific hardgrounds showing excess Ir¹⁷.

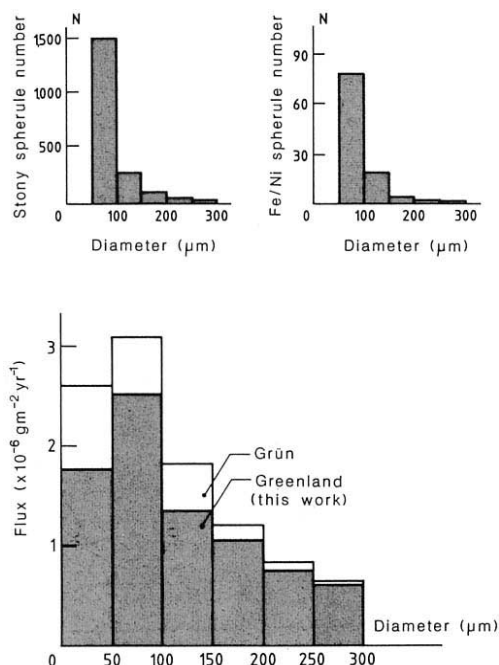


Fig. 2 Size- and mass-frequency distribution of Greenland micrometeorites. The two top histograms give the size distributions of chondritic-stony spheres and oxidized Fe/Ni spheres, respectively. The two lower histograms represent the micrometeorite flux of Grün *et al.*⁴ (solid line) measured at 1 AU, and the flux of the Greenland extraterrestrial dust (shaded area).

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Stratospheric sulphate production and the photochemistry of the Antarctic circumpolar vortex

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Recent measurements of enhanced ozone depletion in springtime in the Antarctic circumpolar vortex¹ were accompanied by observations of unusual behaviour of stratospheric aerosol². Enhanced concentrations of condensation nuclei (CN) were observed above 20 km. Some photochemical models predict elevated hydroxyl radical (OH) concentrations in the vortex. Here I point out that high OH concentrations provide a sufficient source of CN through reaction with carbonyl sulphide (OCS) to account for the observed increase in CN mass. Other mechanisms for CN enhancement are inconsistent with timescales or the vertical structure inferred from aerosol observations. The CN enhancement provides partial support for photochemical models of ozone depletion in the vortex.

Stratospheric aerosols were observed over McMurdo station² in two size ranges, radius $r > 0.01 \mu\text{m}$ and $r > 0.15 \mu\text{m}$ corresponding to newly formed CN and optically active aerosol (OA) respectively. During the period of observation, November 1985, the vortex shifted so that McMurdo station was periodically within and outside the vortex. Observations on 5 November outside the vortex indicated normal behaviour for OA, with a broad peak of 2 cm^{-3} near the tropopause and a gradual if erratic decline by an order of magnitude to 25 km. CN declined from a concentration of $\sim 1,000 \text{ cm}^{-3}$ below the tropopause to $\sim 5 \text{ cm}^{-3}$ at 25 km, a value similar to background mid-latitude measurements. The behaviour for both components was quite different when observations were made on 9 November within the vortex. OA fell from normal values at the tropopause to undetectable levels ($< 2 \times 10^{-2} \text{ cm}^{-3}$) at 25 km. In contrast, the CN were enhanced above 20 km, including a sharp peak of

50 cm^{-3} at 23 km with a half-width of about 2 km. This peak lay above the point of maximum ozone depletion (from observations outside the vortex). Similar CN behaviour was observed in 1983³, 1984 and 1986⁴.

These Antarctic observations resemble events at northern latitudes. Layers of enhanced CN were observed over Laramie, Wyoming each year from 1979-82 by Hofmann and Rosen⁵, who ascribed them to a north polar springtime source perhaps related to increased solar or photochemical activity. In early spring 1983, the OAs observed within the northern polar vortex were a factor of 10 lower than concentrations outside⁶. Enhanced CN were observed over Laramie in 1983 coincident with the depletion of OA in the northern polar region, shortly after a north polar stratospheric warming. Hofmann and Rosen³ have recently ascribed these northern-latitude observations to evaporation of OA at high latitudes followed by transport and condensation of sulphuric acid vapour to CN at lower latitudes. Here I argue that the Antarctic CN enhancement originates by a different mechanism.

The Antarctic observations may be interpreted in terms of a model for the sources and sinks of stratospheric aerosol, composed largely of H_2SO_4 . During periods of low volcanic activity, these aerosols originate in continuous emissions of OCS by biota and by anthropogenic sources at the Earth's surface⁷. OCS, with a lifetime of several years in the troposphere, and subject to little washout, can migrate to the stratosphere. Biotic CS_2 may also be converted to OCS in the troposphere. In addition, episodic large volcanic emissions may contribute SO_2 directly to the stratosphere. But the period following the 1982 El Chichon eruption was relatively quiet so that the 1985 CN concentration at 23 km may be interpreted as originating in background sources⁸.

Stratospheric aerosol may be produced from OCS by a variety of sequences⁹⁻¹³ initiated by photolysis



reaction with hydroxyl radicals



or with atomic oxygen



At mid-latitudes, reaction (1) is the primary stratospheric sulphate source, becoming rapid above 25 km, with lifetimes for OCS of less than a year⁹. Sulphate is produced at the rate of a few molecules per cm^3 per second. The significance of reaction (2) has been problematic in recent analyses due, in part, to large differences among measurements of the relevant rate constant. These ambiguities appear to be resolved by recent measurements by Wahner and Ravishankara¹⁴ who find a rate constant $k_2 = 2 \times 10^{-15} \text{ cm}^3 \text{ s}^{-1}$ with little temperature dependence. For estimated daytime mid-latitude OH concentrations of $\sim 10^6 \text{ cm}^{-3}$ at 25 km, the timescale for OCS removal by (2) may be ten years. Overall, reaction (2) is a secondary source of H_2SO_4 in the stratosphere near 25 km. Reaction (3) may compete with reaction (2) but uncertainties in the rate constant¹⁵ and the $[\text{O}]/[\text{OH}]$ ratio obscure the significance of this reaction.

Now consider the springtime Antarctic observations. During winter, descending motions or sedimentation in the vortex deplete OA, leading to the observed 'aerosol hole'¹⁶. To interpret the CN observations, we shall assume that the 23-km CN layer is a product of vortex springtime photochemistry, with a formation timescale of less than two months. An increased source for CN within the vortex must be sought. Crutzen and Arnold¹⁷ suggest that OH concentrations are enhanced in the vortex due to condensation of nitric acid, removing odd nitrogen species from the vortex gas. Depletion of odd nitrogen through HNO_3 condensation is also a feature of other photochemical models of the vortex^{16,18,19}, although Crutzen and Arnold project higher OH concentrations than do other investigators. Depleted odd nitrogen and enhanced OH increase the efficiency of catalytic

removal of O_3 by Cl. In recent years, 30-mb (23-km) temperatures averaged between 70 and 80° S have measured below 190 K until mid-September²⁰. Temperatures may have remained below 200 K over McMurdo at 30 mb until early October in 1985². These values indicate that nitric acid may reside in condensed phase for 1–2 months beyond early August^{17,18}. Removal of condensed nitric acid through sedimentation¹⁶ could maintain high OH conditions even longer.

Crutzen and Arnold estimate noontime stratospheric OH concentrations of nearly 10^7 cm^{-3} at 22 km in the vortex for 1–2 months from early August. Reaction (1) is slowed by the severe zenith angle, and reaction (2) becomes the largest photochemical sink for OCS in this region. Subsequent conversion of SH and other products to H_2SO_4 appears to be rapid under vortex conditions^{12,13} so the rate of H_2SO_4 production increases during springtime compared to the extra-vortex gas.

If OH is enhanced in the vortex, production of vapour phase H_2SO_4 and formation of CN will also be enhanced. Because the coagulation timescale for 50 CN cm^{-3} is not much less than a year, we can expect CN to survive for months as long as temperatures remain low enough to prevent evaporation. We might then assert that the observed 23-km peak provides evidence of such OH enhancement, presenting partial support for photochemical models of the vortex gas. Two questions must be answered first: can reaction (2) produce sufficient CN in the required time (1–2 months); and are other explanations for the 23-km peak excluded?

A critical parameter which must be estimated is the polar OCS concentration. Mid-latitude values²¹ of 200–500 p.p.t. have been measured at 21 km, which are similar to 15-km measurements and to tropospheric values. The concentration is still 14–18 p.p.t. at 31 km where the photolysis lifetime is about a month. A value of 250 p.p.t. has been measured at 60° S near 11.5 km²². Little seasonal or latitudinal variation is noted in the OCS column from the Equator to 48° N in the stratosphere²³ and the global surface distribution is quite uniform. These measurements are in fair agreement with model calculations²⁴ which indicate an OCS concentration of ~200 p.p.t. at 23 km near 80° S.

The path and timescale for transport of OCS to 23 km over McMurdo are uncertain. The appearance of the OA 'hole' suggests that descending motions in the vortex may contribute air to the 23-km region from higher altitudes where OCS is depleted by photolysis. Without direct measurements the OCS concentration will remain uncertain. We shall use the model OCS abundance of 200 p.p.t., recognizing that it represents an upper limit.

The CN are defined as particles with $r > 0.01 \mu\text{m}$, but smaller than OA, which are absent in any event. We shall assume that CN are spherical, 68% H_2SO_4 droplets with a single radius, $r = 0.02 \mu\text{m}$, corresponding to a weighted mean radius of the true distribution. If $[OCS] = 200 \text{ p.p.t.}$, a diurnal average OH concentration of $4 \times 10^6 \text{ cm}^{-3}$ will produce 50 cm^{-3} CN from reaction (2) in less than two months. Only a small fraction of OCS is lost over that period. Furthermore, enough H_2SO_4 vapour is formed to maintain saturation vapour pressures at the vortex temperatures observed in November²⁵.

Other possible explanations for the enhanced CN include (i) upward transport of CN, (ii) condensation of CN during downward transport and cooling of H_2SO_4 vapour, (iii) reduced loss of CN by coagulation due to the absence of OA surfaces, (iv) transport of H_2SO_4 to McMurdo by intrusion of extra-vortex air, and (v) an evaporation–condensation sequence involving loss of OA leading to formation of CN from the same mass.

Upward transport is an unlikely source of the CN layer as enhanced levels of CN are not observed between 10 and 20 km. Downward transport and cooling of H_2SO_4 vapour could enhance CN concentrations, if peak H_2SO_4 vapour concentrations are available above 30 km, as mid-latitude models predict²⁶. Depending on the thermal history of the vortex air, homogeneous, heteromolecular nucleation of such vapour in

winter or early spring may have been fast and highly temperature-dependent²⁷. Subsidence is strongest in early winter and may be sufficiently rapid^{16,19}, to provide the requisite H_2SO_4 vapour. Future detection of an early-developed, long-lived CN layer would support this hypothesis. But given efficient coagulation by collision, such early CN condensation would lead to measurable numbers of OA by November, which are not observed. Furthermore, neither observed nor calculated H_2SO_4 vapour concentrations above 30 km are high enough to account for the observed increase in CN mass²⁶. There is no evidence of remnant H_2SO_4 vapour above 20 km from the El Chichon eruption⁸.

Lack of OA surfaces for attachment and coagulation of CN will slow CN removal in the vortex but the timescale for this process is of the order of a year or rather longer than a month. Lack of OA has little effect on H_2SO_4 vapour pressure and CN formation rate because CN provide an equivalent surface area for condensation under Antarctic conditions. Furthermore, CN and OA do not exhibit strictly complementary behaviour above 20 km. At lower altitudes, their behaviour appears to be more closely coupled and OA concentrations are high enough to play a part in CN removal.

We have not performed trajectory analysis to exclude intrusion of extra-vortex air as a source of CN. However, both the low ozone and the low OA concentrations observed on 9 November are consistent with the absence of such air parcels.

A local evaporation–condensation sequence involving the missing OA mass, proposed previously to interpret the 1983 McMurdo observations³, is an unsatisfactory explanation, at least for the 1985 data. The vortex gas temperature would have had to exceed, at some time, the -30°C values observed outside the vortex on 5 November when OA concentrations were high. As we noted, lack of OA in the vortex is probably due to subsidence or sedimentation. In that case, as our arguments have shown, the limiting factor on CN production appears to be availability of H_2SO_4 vapour. So an increased condensation rate due to low temperature alone cannot produce sufficient CN, although low temperatures may play a role in conjunction with an enhanced source. These arguments are not conclusive but they suggest some of the problems with dynamical explanations of the aerosol behavior. A time series of aerosol observations could distinguish between these models as subsidence is an early winter phenomenon^{16,19} and enhanced OH occurs much later.

The structure of the aerosol profile is consistent with reaction (2) as a major source in the vortex. The calculations of Crutzen and Arnold indicate increasing OH with increasing altitude in the vortex. But OCS decreases sharply above 25 km⁹. Near or below 20 km, extinction measurements suggest that CN may coagulate on, or nucleate, cloud particles over much of the period. Thus, OAs, but not CN, increase rapidly between 20 and 15 km. So the CN increase would be rather restricted in altitude. Other molecules such as the hydroperoxyl radical (HO_2) are also enhanced in the photochemical models and these species may also play a role in vortex sulphur chemistry. But linking the detailed behaviour of the CN to the photochemical models is premature as the models cannot yet reproduce the ozone profiles. For example, the CN peak lies above the point of maximum ozone depletion, a feature which requires exploration. This observation may be explained by the lower temperatures at altitudes below 20 km which could extend the period for ozone removal⁴.

Elevated OH also may have led to the high concentrations of condensation nuclei observed near 23 km over McMurdo in October 1983³, 1984, and 1986⁴ although the 1983 observation is complicated by the presence of sulphur from El Chichon. If further investigation confirms CN enhancement as a short-lived phenomenon which develops in springtime, then the enhanced CN may be taken as a validation of one element of the photochemical models for ozone depletion.

Antarctic observations of OCS and H_2SO_4 vapour and modelling of Antarctic atmospheric sulphur chemistry are also of significant interest. The OCS concentrations used in our calculations may be our most vulnerable assumption.

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Evidence for endothermic quasicrystalline–crystalline phase transitions in Al_6CuMg_4

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Since the discovery of quasicrystalline phases with icosahedral symmetry in an Al–Mn system by Shechtman *et al.*¹, a variety of other quasicrystals have been reported. Recently a new class of quasicrystalline alloys has been predicted and produced^{2,3} (two prominent members of this class are Al_6CuLi_3 and Al_6CuMg_4) which has resulted in the production of large quasicrystals. Large (~millimetres) quasicrystals of Al_6CuLi_3 have already been reported⁴. We have used electron microscopy, differential scanning calorimetry (DSC) and X-ray diffraction to investigate the quasicrystalline–crystalline phase transition in the rapidly solidified Al_6CuMg_4 alloy system. We present the first evidence that an endothermic quasicrystalline–crystalline transition occurs in this system at ~340 °C. This is in contrast with the exothermic crystallization of quasicrystalline $\text{Al}_{86}\text{Mn}_{14}$ (ref. 4) and suggests that certain quasicrystalline phases may be stable (as recently observed⁵ in the Al_6CuLi_3 system). This study also reveals that a close structural relation exists between crystalline and quasicrystalline phases of Al_6CuMg_4 .

The stoichiometric compound (Al_6CuMg_4) was prepared by melting constituent elements in pure form (99.99%) under argon atmosphere in a graphite crucible. Ribbon samples for this study, of about 5-mm width and thickness 30 μm , were obtained by

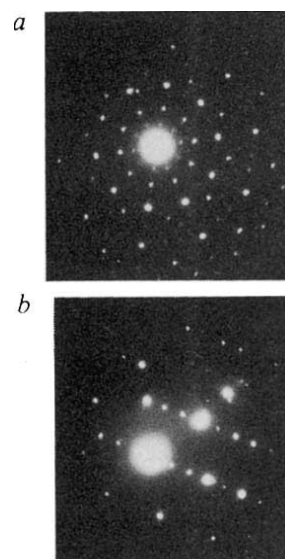


Fig. 1 Electron diffraction patterns, of as-grown sample exhibiting fivefold symmetry (a) and of heat-treated quasicrystalline sample (b).

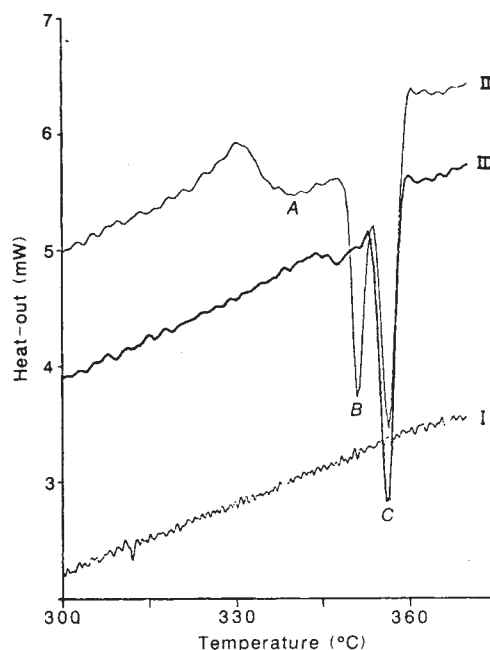
rapid solidification by the usual melt spinning method. The quasicrystalline character of these samples was confirmed by transmission electron microscopy (TEM) observations. Electron diffraction patterns showing fivefold (Fig. 1a), twofold and threefold symmetries (characteristic of the quasicrystalline phase) were obtained from the same nodule. X-ray analysis of the sample was done to confirm that it was of single phase and the aluminium peak was not present. Quasicrystalline grains in this ribbon were found to be contiguous without any matrix of the f.c.c. aluminium phase and so it is extremely brittle.

Three DSC measurements were performed: one on the original crystalline material (from which quasicrystalline ribbons were prepared), the second on 'as-grown' quasicrystalline phase, and the third on quasicrystal samples that were heated *in situ* to 400 °C, then cooled to 30 °C before the DSC scans were made. The results for these three measurements are identified by the symbols I, II and III, respectively, in Fig. 2. We performed the measurements several times with different portions of materials to confirm the repeatability of the results. All the DSC measurements were done on Mettler TA3000 thermal analysis system and the system was calibrated with pure indium.

Above 400 °C, the results for all three are similar, in that there is a strong endothermic peak (due to melting) at 474.9 °C and enthalpy change is 1.6 Kcal mol⁻¹. But, below 360 °C the spectra are very different, as shown in Fig. 2. Whereas for the original crystalline material, I, the heat-out against temperature curve shows no peaks, for the as-grown quasicrystalline phase, II, we see a broad endothermic structure at 340 °C (A) followed by two sharp peaks at 351 °C (B) and 356.5 °C (C); the corresponding enthalpy changes are 15.5, 10.5 and 17.6 cal mol⁻¹. For the heat-treated samples, III, there is only a sharp endothermic peak at 356.5 °C coinciding with the peak C of II, and neither the broad structure A nor the peak B is seen. The peak C is connected with a reversible (polymorphic) crystalline phase transition because it was observed during cooling also.

To elucidate the nature of A and B, the as-grown sample was kept isothermally in DSC at A (340 °C) for 1 h. Subsequent DSC scans through higher temperatures did not show the presence of B. In view of this, A was used to study the kinetics by a non-isothermal method (based on the peak temperature–heating rate relation) and activation energy for crystallization was found to be 62 Kcal mol⁻¹, which is similar to that observed in an Al–Mn system (53 Kcal mol⁻¹). It was observed that the

Fig. 2 Recorded DSC data: I, for original crystalline material (10 mg of sample); II, for as-grown quasicrystalline phase (5mg of sample); and III, for heat-treated quasicrystalline sample.



separation of electron diffraction spots of the as-grown quasicrystalline sample is related to the 'golden ratio' (Fig. 1a) whereas those of the heat-treated (at 340 °C) sample exhibit a periodicity (Fig. 1b), which confirms the crystallization.

X-ray measurements were done with both high-resolution angle-dispersive diffraction (in Siemens D-500 system) and energy dispersive X-ray diffraction (EDXRD)⁶ techniques. EDXRD patterns of quasicrystalline phase and transformed crystalline phase are shown in Fig. 3. Some of the diffraction peaks, whose d -values were measured in Siemens D-500 system, are not resolved in this pattern because of poor resolution of EDXRD technique. The d -values obtained from the diffraction peaks of the quasicrystalline phase of this alloy are: 2.46, 2.33, 2.06, 2.03, 1.99, 1.79, 1.44, 1.37, 1.26, 1.22, 1.16, 1.08; whereas corresponding d -values obtained in quasicrystalline Al_6CuLi_3 alloy are 2.39, 2.27, 2.00, 1.96, 1.93, 1.75, 1.40, 1.33, 1.21, 1.19, 1.14 and 1.06. The ratio of the d -values of these two alloys is 1.03; this corresponds with the ratio of the lattice constants (namely 14.35 Å and 13.91 Å) of the corresponding crystalline phase ($\text{Im}\bar{3}-T_h$) of these alloys. The diffraction pattern of the heat-treated sample corresponds very well with that of the crys-

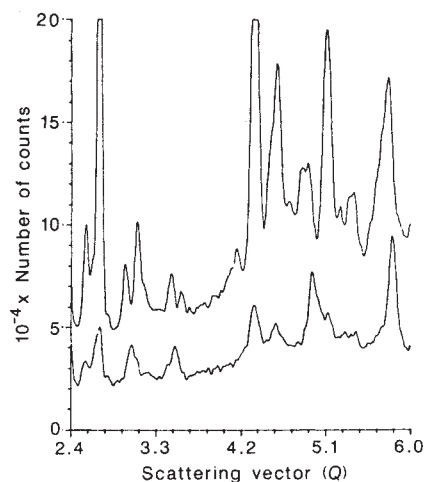


Fig. 3 EDXRD pattern of as-grown (lower) and heat-treated (upper) quasicrystalline sample. For clarity the pattern of heat treated sample is shifted upward.

talline $\text{Mg}_{32}(\text{Al}, \text{Cu})_{49}$ (with Al:Cu = 6:1) phase⁷ except for the minor changes in the intensity ratios, which are due to fractional occupancy of empty lattice sites. The d -values obtained in the transformed sample are: 3.85, 3.60, 3.39, 3.06, 2.82, 2.63, 2.46, 2.39, 2.33, 2.24, 2.12, 2.03, 1.99, 1.96, 1.92, 1.89, 1.82, 1.77, 1.67, 1.55, 1.53, 1.51, 1.48, 1.45, 1.43, 1.39, 1.37, 1.32, 1.30, 1.28, 1.24, 1.23, 1.10, 1.09, etc. We could not find any trace of the second phase in the diffraction patterns of both as-grown and heat-treated quasicrystalline samples.

From the analysis (details will be presented elsewhere) of X-ray data, we have found that the quasilattice constant (a_R) of this quasicrystalline alloy is 5.21 Å. The lattice constant a of crystalline phase (14.35 Å) and the quasilattice constant was found to obey $a = a_R(4 + 8/S^{1/2})^{1/2}$, which was predicted by a theoretical model⁸. This result confirms that the local structure of quasicrystalline and crystalline phase of this alloy are not very different⁹ and is consistent with the fact that enthalpy change (15 cal mol⁻¹) in this phase transition $\ll$ that (560 cal mol⁻¹) reported⁴ in the crystallization of the quasicrystalline Al-Mn alloy.

It is not possible⁷ to obtain single-phase crystalline Al_6CuMg_4 alloy even after prolonged heat treatment. The DSC scan of original crystalline material was done to observe whether endothermic peak occurs at the same temperature owing to peritectic melting. Absence of any such peak in the scan confirms that the peaks A and B are indeed due to the quasicrystalline-crystalline phase (both are found to be single phase) transformation. Peak C is not seen in the DSC scan of crystalline material because the high temperature phase is also quenched in it. In conclusion, we have found that the quasicrystalline phase of the aluminium-rich ternary alloy Al_6CuMg_4 is more stable than the crystalline phase (isostructural to $(\text{Al}, \text{Cu})_{49}\text{Mg}_{32}$) of this material. This suggests that, by judicious choice of cooling rate, it should be possible to grow large-sized quasicrystals, akin to what has been found possible⁵ in the aluminium-rich¹⁰ alloy Al_6CuLi_3 system.

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Paramagnetic shift probes in high-resolution solid-state NMR

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Small additive shifts due to changes in the local environment of the resonating nucleus have been observed in magic-angle spinning nuclear magnetic resonance (MASNMR) studies of several classes of solid. The ^{29}Si resonances in zeolites, for example, shift by approximately 5 p.p.m. per aluminium next-nearest-neighbour^{1,2}, and similar effects have been observed in ^{31}P MASNMR spectra of $\text{Zn}_{3-x}\text{Mg}_x(\text{PO}_4)_2$ solid solutions³. The use of paramagnetic ions to induce large chemical shifts is well established in solution NMR⁴ and has also been observed for molecular structures in the solid state⁵. Here we describe a ^{119}Sn study of the pyrochlore solid solution, $\text{Y}_{2-x}\text{Sm}_x\text{Sn}_2\text{O}_7$, and show for the first time the use of paramagnetic shifts in MASNMR to probe the structures of continuous solids.

$\text{Y}_2\text{Sn}_2\text{O}_7$ and the lanthanide-tin oxides ' $\text{Ln}_2\text{Sn}_2\text{O}_7$ ' adopt the cubic pyrochlore structure^{6,7}, which is characterized by two parameters: the cell constant, which depends upon the size of the lanthanide ion, and the x parameter, which defines the location of one of the two independent oxygen atoms. There is one tin site in the asymmetric unit, point symmetry $3m$, and the stereochemistry around the tin can vary from a regular to an axially compressed octahedron. The ^{119}Sn MASNMR spectra ($I = \frac{1}{2}$, abundance = 8.6%) of the diamagnetic pyrochlores ($\text{Ln} = \text{La}, \text{Y}, \text{Lu}$) show single isotropic resonances, as expected, which occur at -642, -582 and -563 p.p.m. (from Me_4Sn) respectively (Fig. 1). These shifts are found to change smoothly with variations in the ionic radii of the Ln^{3+} ions. Both ^{139}La and ^{175}Lu are $I = 7/2$ quadrupolar nuclei of high natural abundance, and this is the cause of the relative broadening of the ^{119}Sn resonances from the La and Lu stannates compared with the yttrium compound. The spectrum from pure $\text{Y}_2\text{Sn}_2\text{O}_7$ is sufficiently well resolved to reveal satellite resonances, presumably due to coupling between ^{119}Sn and ^{117}Sn ($I = \frac{1}{2}$, 7.6% abundant) as has been recently observed in a series of stannates with the perovskite structure (N. J. Clayden, C.M.D. & A. Fern, unpublished data).

Figure 2 (top) shows a spectrum of $\text{Sm}_2\text{Sn}_2\text{O}_7$ with an isotropic resonance at -92 p.p.m. A comparison of the $\text{Sm}_2\text{Sn}_2\text{O}_7$ spectrum with those of the diamagnetic $\text{Ln}_2\text{Sn}_2\text{O}_7$ compounds reveals two important changes: the isotropic resonance has shifted ~500 p.p.m. downfield, and the anisotropy ($\Delta\sigma = \sigma_{33} - \frac{1}{2}(\sigma_{22} + \sigma_{11})$, where the σ_{ii} are the orthogonal tensors estimated by an analysis of the sideband intensities⁸) has increased in magnitude from ~-20 p.p.m. to ~-240 p.p.m. The origin of these shift changes can be ascribed to interactions between the unpaired electrons of the paramagnetic Sm atoms and the ^{119}Sn nuclei. Both scalar (Fermi contact) and dipolar (pseudo-contact) terms are likely to contribute to this interaction⁹, and the latter term is probably the cause of the increased anisotropy observed. An additional effect of these interactions in $\text{Sm}_2\text{Sn}_2\text{O}_7$ is a greatly reduced spin-lattice relaxation time of the ^{119}Sn nuclei.

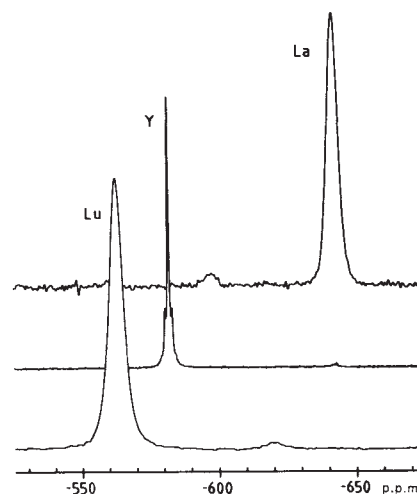


Fig. 1 The ^{119}Sn MASNMR spectra of the three diamagnetic stannates, $\text{La}_2\text{Sn}_2\text{O}_7$ (La), $\text{Y}_2\text{Sn}_2\text{O}_7$ (Y) and $\text{Lu}_2\text{Sn}_2\text{O}_7$ (Lu). The isotropic resonances are shown with chemical shifts relative to Me_4Sn .

Figure 2 shows ^{119}Sn MASNMR spectra from preparations with stoichiometry $\text{Y}_{2-x}\text{Sm}_x\text{Sn}_2\text{O}_7$, $x = 0.0, 0.2, 0.5, 1.0, 1.5$ and 2.0 , collected with a short delay time (0.5 s) between pulses. By using a short delay time, signals from nuclei with long relaxation times are saturated and it is possible to increase the relative intensities of signals from nuclei with fast relaxation times. This can be particularly useful when the latter are in low concentrations in the solid. A comparison of the MASNMR spectrum of the $x = 0.2$ stoichiometry with that of the pure $\text{Y}_2\text{Sn}_2\text{O}_7$ ($x = 0.0$) reveals an additional peak at -492 p.p.m. (resonance B), and another new peak at -404 p.p.m. (resonance C) is just discernible. Peak G, further downfield, with its prominent associated sidebands, is due to $\text{Sm}_2\text{Sn}_2\text{O}_7$. The intensities of resonances B and C increase with increasing x and at the same time more peaks become visible; when $x = 1.0$, five new peaks (B, C, D, E and F; Fig. 3) can be seen. These resonances (B to F) cannot be directly attributed to either end-member compounds and

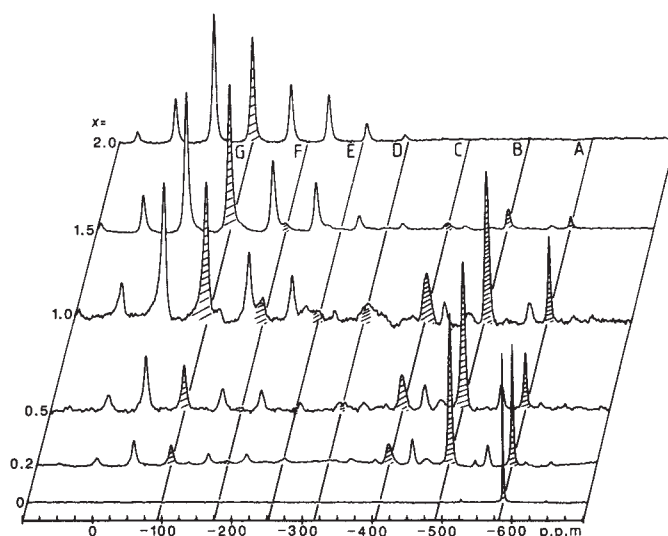


Fig. 2 ^{119}Sn MASNMR spectra from samples with varying stoichiometries $\text{Y}_{2-x}\text{Sm}_x\text{Sn}_2\text{O}_7$, $x = 0.0$ ($\text{Y}_2\text{Sn}_2\text{O}_7$), $0.2, 0.5, 1.0, 1.5$ and 2.0 ($\text{Sm}_2\text{Sn}_2\text{O}_7$). The isotropic resonances A to G are shaded and each is traced as a function of composition. The remaining peaks are spinning sidebands.

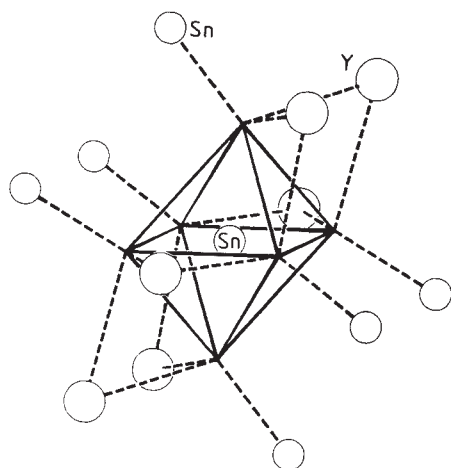


Fig. 3 The local environment of the tin in $Y_2Sn_2O_7$ showing the next-nearest tin and yttrium ion neighbours. The full lines represent the SnO_6 octahedra with the oxygens at the apices.

would appear to stem from changes in the local tin environment when samarium substitutes for yttrium in the $Y_2Sn_2O_7$ structure. X-ray powder diffraction patterns at intermediate stoichiometries confirm that no new phases are present, but the main lines of $Y_2Sn_2O_7$ and $Sm_2Sn_2O_7$ are shifted very slightly, indicating the formation of a limited, mutual solid solution in both end-members. The changes in the lattice parameters are too small to facilitate a reliable determination of the composition limits of the solid solutions.

An examination of the local coordination of tin in the structure of $Y_2Sn_2O_7$ (see Fig. 3) indicates that this site has six next-nearest neighbour Y atoms (that is, those linked by Sn-O-Y connections) and six next-nearest tin atoms. Hence the substitution of Sm for Y can give rise to seven different local environments around the tin. Our interpretation of the spectra is that these local environments give rise to the seven resonances A to G. Peak A must represent a tin with no next-nearest Sm atoms and peak G a tin with six next-nearest Sm atoms. Of the intermediate resonances, peak B (at -492 p.p.m.) arises from a single Sm substitution and in the same way the resonances C, D, E and F result from the following local tin environments: $Sn(OY)_4(OSm)_2$, $Sn(OY)_3(OSm)_3$ and $Sn(OY)_2(OSm)_4$ and $Sn(OY)(OSm)_5$, respectively. On the basis of this allocation of resonances, and obtaining spectra collected under conditions of complete relaxation of all tin atoms, it is possible to use the intensities of peaks A to C to determine the limit of solid solution of $Sm_2Sn_2O_7$ in $Y_2Sn_2O_7$. The results of such experiments indicate the limiting value of $Y_{1.85}Sm_{0.15}Sn_2O_7$. The corresponding value for yttrium in the samarium phase is significantly smaller (approximately $Sm_{1.97}Y_{0.03}Sn_2O_7$).

Similar additive shifts have been obtained on doping samarium into $La_2Sn_2O_7$ and $Lu_2Sn_2O_7$. The shift caused by the paramagnetic ions is, as expected, sensitive to the Ln-Sn distance, and as this distance decreases from La to Y to Lu, the shift increases. Table 1 shows how these shifts, which correspond

Table 1 ^{119}Sn shifts caused by substitution of Sm ions into the La, Y and Lu pyrochlores

Parent compound	Ln-Sn distance (Å)	Dopant ion	Shift (p.p.m.)
$La_2Sn_2O_7$	3.78 (ref. 10)	Sm	+77
$Y_2Sn_2O_7$	3.67 (ref. 10)	Sm	+90*
$Lu_2Sn_2O_7$	3.64 (refs 6, 7)	Sm	+96

* When Y is doped into $Sm_2Sn_2O_7$ the corresponding (peak G to peak F) shift is 80 p.p.m. (the Ln-Sn distance = 3.71 Å)^{6,7}.

to that of peak A to B in the $Y_2Sn_2O_7$ case (see Fig. 2), vary with the Ln-Sn distance.

In view of the small chemical shift effects that are often observed in solids and the consequent difficulty in their interpretation, it is to be hoped that large shifts of the type observed in this study will be of value in the interpretation of high-resolution NMR spectra from a wide range of continuous solids.

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Interferometric radar measurement of ocean surface currents

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We describe a new method of measuring surface currents using an interferometric synthetic aperture radar. An airborne implementation has been tested over the San Francisco Bay near the time of maximum tidal flow, resulting in a map of the east-west component of the current. Only the line-of-sight component of velocity is measured by this technique. Where the signal-to-noise ratio was strongest, statistical fluctuations of less than 4 cm s^{-1} were observed for ocean patches of $60 \times 60 \text{ m}$.

Microwaves interact mainly with the topmost few centimetres of the ocean surface. Rice¹ has shown that, for a gently undulating surface between dielectric and air, only one spatial Fourier component of the surface is dominant in reflecting an electromagnetic wave. This spatial component must be aligned at right angles to the line-of-sight direction and its projected wavelength in this direction must equal half the radio wavelength (the Bragg condition). If the surface is in motion, as would be the case for water waves, then a Doppler shift would be observed according to the phase velocity of the water waves.

Valenzuela² has devised a model of the ocean surface wherein longer waves (the swell) modulate the shorter Bragg waves. Even though the synthetic aperture radar detects directly only the shorter waves, the swell is visible in the images through this mechanism. The observed velocity of an element of the reflecting surface is thus the sum of the orbital motion of water particles from the swell and the phase velocity of the Bragg waves. Added to these, of course, are the surface portion of any underlying ocean currents that may exist.

Gonzales *et al.*³ have observed such motion with a radar over the mouth of the Columbia River by estimating the Doppler shift of all the combined echoes across the radar beam. Raney⁴ has described a two-antenna scheme for detecting motion where stationary targets are cancelled, leaving only the moving ones to be seen in the radar image.

Our method is an extension of Raney's which also uses a conventional synthetic aperture radar system augmented with

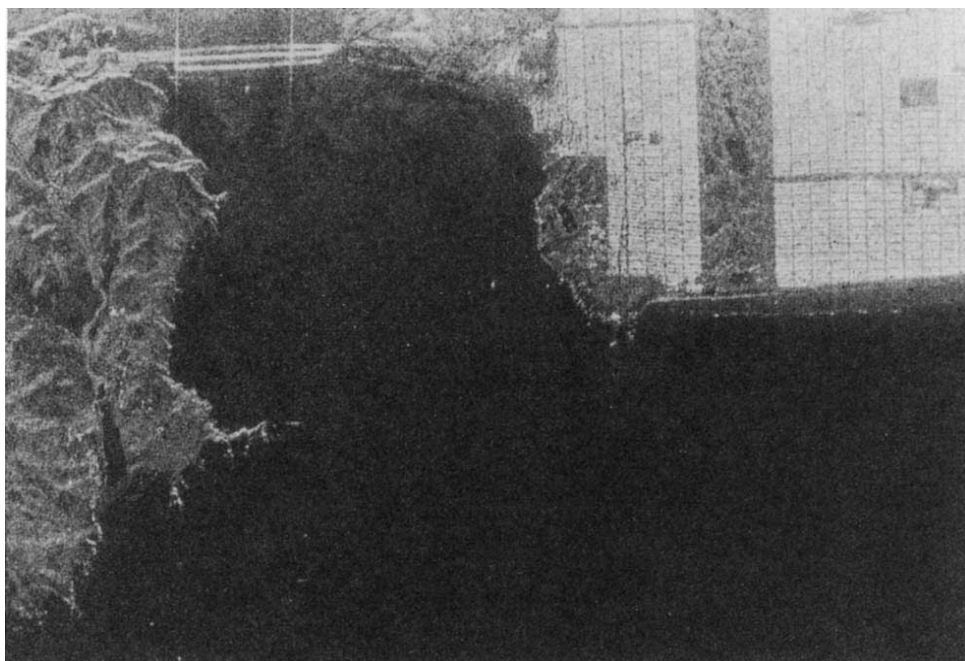


Fig. 1 Conventional synthetic aperture radar image of the San Francisco Bay region. Area imaged is $\sim 11 \times 11$ km; resolution is $\sim 11 \times 11$ m. Image brightness is proportional to backscatter amplitude.

two antennas, spaced along the radar velocity direction (along-track). The signals from each antenna are separately processed into two images, which are then combined interferometrically. Motion of the surface causes a phase difference between corresponding picture elements (pixels), which is proportional to the radial distance moved by the resolution element (rezel) in the time required for the rear antenna to move to the position formerly occupied by the forward antenna:

$$\phi = \frac{4\pi u B}{\lambda v} \quad (1)$$

where λ is the radar wavelength, B is the distance between antennas, v is the radar velocity and u is the line-of-sight velocity component of the rezel.

The phase of an echo from a given rezel changes continuously with time as the radar moves. Indeed, it is this property which gives synthetic aperture radar its fine resolution in the along-track direction. What then is the single phase of the resulting pixel? It depends, of course, on the signal-processing algorithm which produces the image. For most processing schemes it is the phase at the centre of the observation time. For others, it is the phase at closest approach. In any event, if the two images are processed in the same way, the phase difference of equation (1) is obtained.

For the above, we have assumed that the two antennas are collinear with the radar motion. If this is not the case, then there will almost always be a phase difference between corresponding pixels, as one of the antennas will, in general, be closer. This is a fundamental problem; the radar cannot distinguish between a slight sideways drift of its platform and radial motion of the whole ocean. We have resolved this problem by including land in the scene and use the stationarity of the land to estimate the radar attitude. In the absence of land for calibration, accurate knowledge of the radar attitude is necessary. For our system, a yaw error of 0.01 degrees will cause an error in the current estimate of 4 cm s^{-1} .

We have tested this method with an L-band radar (wavelength = 24.5 cm) on board a NASA CV990 aircraft. One antenna was mounted near the rear cabin door, the other near the front, forming a baseline of 18.5 m. For this implementation, the transmitter was always connected to the rear antenna and signals were received simultaneously with both antennas. The

use of only one of the antennas for transmission reduces the phase difference of equation (1) by a factor of two.

The test flight was heading south over the San Francisco Bay region at an altitude of 26,000 ft, a velocity of 238 m s^{-1} and a drift angle less than 0.2° . Data were acquired 30 min after the predicted time of maximum tidal ebb current of 2.6 m s^{-1} (ref. 5).

Figure 1 is the standard synthetic aperture amplitude image of the scene, where the brightness of each pixel is proportional to backscattering amplitude. Resolution along-track (north-south) is about 12 m. Resolution in range (across-track) varies across the image; 11 m is typical. An area of $\sim 11 \times 11$ km is imaged. The Golden Gate Bridge (near the top) appears as three images because of the multiple round trip paths available to the radar wave (for a discussion of this effect, see ref. 6). The look angle changes from $\sim 20^\circ$ at near range to $\sim 57^\circ$ at far range.

Figure 3 is the same scene, but pixel phase difference measurements have been added and are shown in colour. Zero motion is presented as cyan with ebb flow in green to yellow; flood

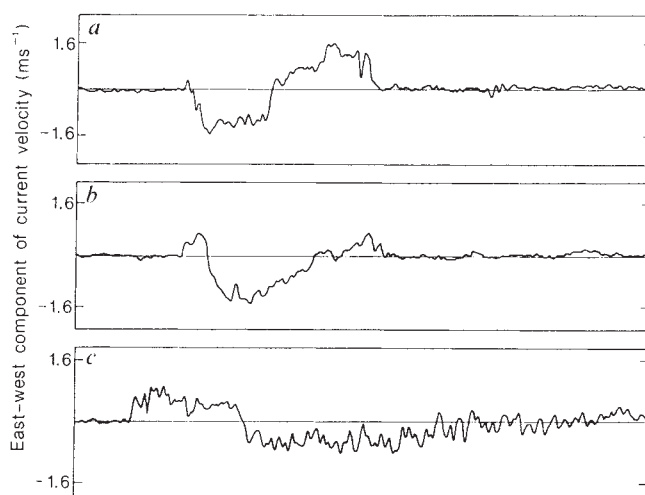
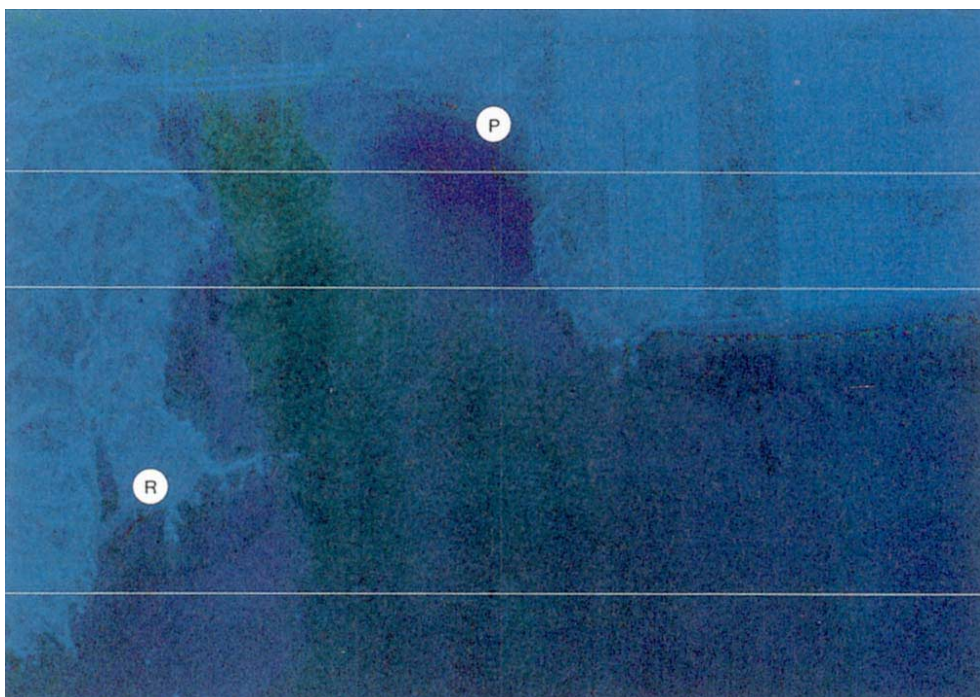


Fig. 2 Plots of measured current velocity (east-west component) corresponding to different incidence angles: *a*, 37° ; *b*, 43° ; and *c*, 53° . Abscissas of plots are shown in white in Fig. 3.

Fig. 3 Same as Fig. 1, but velocity measurements, in colour, have been added.



currents range through violet to magenta. Brightness in Fig. 3 is still proportional to signal amplitude, but because the ocean is less reflective at radar frequencies than land, adjusting the brightness to highlight the ocean results in land portions of the image appearing considerably overexposed. The largest east-west component of tidal flow which we observed was 1.6 m s^{-1} (yellow in Fig. 3). The total current velocity was greater because there was also a north-south component. An eddy with the surprising velocity of 1.6 m s^{-1} was observed near the Presidio (marked P in Fig. 3). Because of the circular nature of an eddy, the maximum velocity is the velocity observed. A larger eddy, of smaller velocity (0.8 m s^{-1}), was seen off Rodeo Cove (marked R). Both eddies appear as purple in this figure.

Our data are somewhat flawed in that well-sampled aircraft attitude angles were not recorded. The aircraft underwent yawing at $\sim 0.25 \text{ Hz}$ which placed a series of corrugations, parallel to the cross-track dimension, in the velocity image. Although the inclusion of land in the image allowed us to remove most of this effect, a series of irregular vertical bars can still be seen, faintly, in the far range in Fig. 3.

Figure 2 is a set of plots of the east-west current component, at different positions across-track. These locations are marked with white lines in Fig. 3. The scales of the plots of Fig. 2 are somewhat different, reflecting the differing line-of-sight projections as the incidence angle changes. For these graphs, areas of 6×6 pixels have been averaged, resulting in surface resolution of $\sim 60 \times 60 \text{ m}$. One can see that the noise level (for the ocean) increases rapidly with incidence angle—a consequence of the fact that the ocean was relatively smooth when these observations were made.

We ascribe the measured currents to tidal flow; little swell is observed in the image. As discussed above, we expected our image to indicate total velocities, that is, the velocity at each point represents the sum of line-of-sight components of the current, the swell, and the Bragg waves. But only the current component of the waves is visible. We rule out the presence of swell because our resolution is fine enough to resolve individual crests and troughs of the swell, which would produce a banded pattern in the velocity image if large swell were present.

The Bragg waves, with a phase velocity of about 0.5 m s^{-1} are well above our threshold sensitivity and, if moving in only one

direction, should cause the entire ocean surface to appear in Fig. 3 at a different 'colour' from the stationary land. We note, though, that the land and ocean, apart from currents, are at the same colour (and hence, velocity). If there were no Bragg waves, we would measure no backscatter at all from the ocean. Thus, we must conclude that each resolution element contains nearly equal Bragg component waves travelling both towards and away from the radar. These two sets interact to form standing waves on the ocean surface. Such standing waves result in pixels unshifted in phase, but still allow interaction between the radar signals and the surface.

Interferometric radar measurements allow acquisition of a new data type useful for studying the ocean surface. We measure directly the velocity associated with each $11 \text{ m} \times 12 \text{ m}$ resolution cell on the ocean—each velocity is the sum of waves, swell and current.

We have mapped the ebb current flowing out of San Francisco Bay to a velocity resolution of $\sim 4 \text{ cm s}^{-1}$; the uncertainty can be reduced by more accurate knowledge of aircraft attitude. We measured a peak value of 1.6 m s^{-1} in the east-west component of ebb flow, which corresponds with an expected 2.6 m s^{-1} from tidal current tables. In addition, two eddy currents of 1.6 m s^{-1} and 0.8 m s^{-1} were seen. Because of radar hardware problems we were unable to obtain data on our east-west leg of the flight, which, in principle, could have allowed us to measure the remaining, perpendicular components of the observed surface currents.

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Pleistocene occupation in arid Central Australia

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Interest in the pattern and rate of human colonization of Australia has been stimulated by the hypothesis that the arid interior of the continent was initially settled as late as 10,000–12,000 yr BP (ref. 1). The failure of several field projects to locate earlier sites, despite systematic searches, has lent support to this view. However, recent archaeological excavations at Puritjarra rockshelter, in Central Australia, have revealed a stratified deposit containing stone artefacts in levels dating to the late Pleistocene. Radiocarbon dates from this site indicate that the central part of the arid zone was settled by 22,000 yr BP, about 12,000 years earlier than previous studies² have shown. These results provide first evidence of occupation of the central desert during the Pleistocene and should now end a decade of scientific speculation about the timing of human settlement in the arid zone^{1,3–5}.

Puritjarra rockshelter is in the Cleland Hills, a series of low hills of Devonian sandstone outcropping in a dunefield, 75 km west of the main MacDonnell ranges (Fig. 1). The site is formed at the base of a small escarpment where a large overhang provides around 400 m² of level, shaded, floor area and which is close to the only permanent water in the hills. Occupation of the site continued until the 1930s when the area was depopulated by the migration of Aboriginal people into missions and ration depots in the western MacDonnell ranges.

Initial analysis of the site has concentrated on stratigraphy and chronology, and on a preliminary examination of associated archaeological material from two 1 m × 1 m pits (designated N9 and N10), representing half of the area excavated. All excavated material was dry sieved in the field using 3-mm and 6-mm mesh. The sieve residues were transported to the museum where the charcoal was floated off and the stone artefacts and bone material removed for analysis.

Four radiocarbon dates have been obtained from Pit N10 (Table 1). For Beta-18882, Beta-18884 and Beta-19901 the material dated was scattered charcoal pieces recovered from the sediment by flotation. Beta-18883 dates a single large lump of charcoal found embedded in layer II sediments during excavation.

The deposit consists of three well-defined stratigraphic layers (Fig. 2). Extending from the present surface (0 cm) to a depth of 42 cm, layer I is a loose, gritty, light brown sand (Munsell colour 5YR 5/8) containing lenses of rockfall, intact hearths, charcoal, flaked stone artefacts, grindstones, ochre and emu egg-shell. It gives evidence of a major increase in occupation of the region during the last one thousand years, a change shown in more detail in other sites^{6,7}.

Evidence for Pleistocene occupation occurs in layer II, which consists of compact, fine, red clayey sand (Munsell colour 2.5YR 5/8). The lowest artefacts were recovered from the middle of this layer (66–77 cm) associated with charcoal dated to 21,950 ±

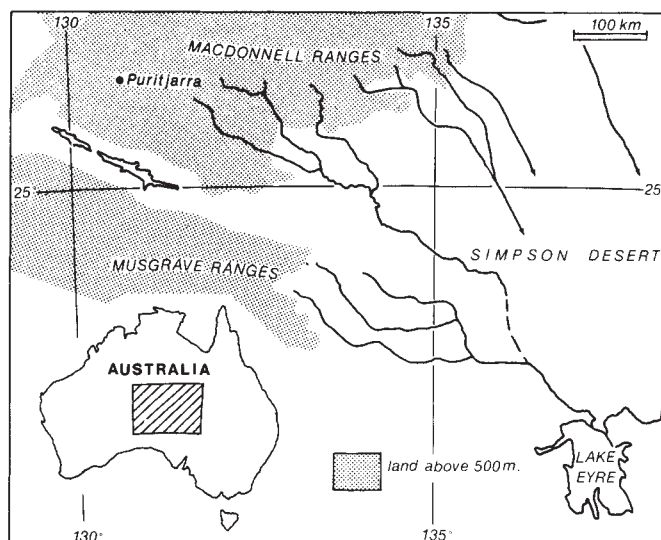


Fig. 1 Map of Central Australia showing the location of Puritjarra rockshelter.

270 yr BP. On present evidence the date of 22,440 ± 1370 yr BP, from the lower part of layer II, predates occupation of the rockshelter.

Layer III (101–212 cm) consists of well-rounded rubble in a matrix of loose, fine dark red sand (Munsell colour 10R 5/8). No definite evidence of occupation was found in Layer III. The full depth of the layer was not established nor was bedrock reached.

The use of Puritjarra rockshelter between 22,000 and 12,000 yr BP was low and is represented by the deposition of only a few artefacts per millennium (Table 2). Generalized palaeoenvironmental data for the period from 25,000–16,000 yr BP indicates widespread drying of lakes, extensive construction of aeolian dunes and an expansion of the arid zone on both northern and southern margins⁸. Studies of pollen and sediments are planned to establish the conditions that prevailed at Puritjarra during this period.

The artefacts from the Pleistocene levels suggest the production of large flakes and large flake implements. However, the presence of a small blade core, and of several parallel-sided elongate flakes from a similar core, show that this early assemblage should not simply be characterized as a large flake industry. A small piece of red pigment was found associated with the basal occupation.

From about 6,000 yr BP the shelter was used more frequently but the period of greatest use occurs in the upper 10–14 cm of

Table 2 The distribution and concentration of flaked stone artefacts in layers I–III, Pits N9 and N10 combined

Layer	Volume (m ³)	No. of artefacts	Total weight (g)	Estimated no. artefacts	
				per m ³	per 1,000 yr
I upper	0.26	1,252	1,981.8	4,815	656
I lower	0.64	860	2,889.9	1,344	210
II upper	0.55	92	2,575.2	167	6
II lower	0.49	—	—	0	0
III	0.55	—	—	0	0

Layer I is arbitrarily divided into an upper part estimated to post date 1,900 BP, and a lower part dating from about 6,000–1,900 BP. Layer II is divided into an upper part containing artefacts and a lower part in which artefacts are absent.

Table 1 Radiocarbon dates

Excavation unit	Depth (cm)	Layer	Sample no.	Date (yr BP)
N10/6	32–42	I	Beta-18882	5,860 ± 150
N10/9	53	II	Beta-18883	12,020 ± 240
N10/11	66–77	II	Beta-19901	21,950 ± 270
N10/13	89–101	II	Beta-18884	22,440 ± 1,370

Depths are given in cm below surface.

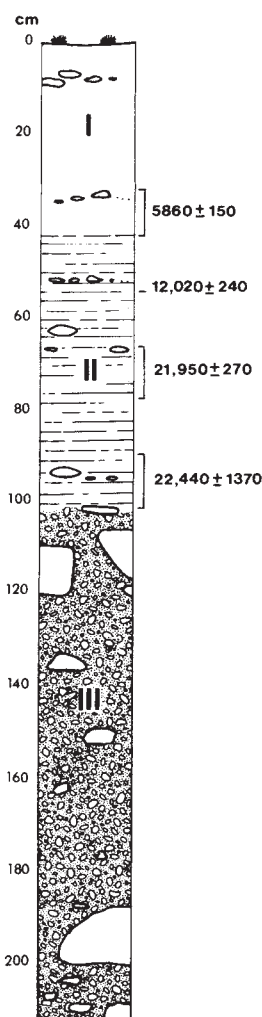


Fig. 2 Schematic stratigraphic diagram showing the location of radiocarbon samples. Layer I is a loose, gritty, light brown sand. Layer II consists of compact, fine red clayey-sand. Layer III consists of sandstone rubble and fine, dark red sand.

layer I, estimated to post-date 1,900 yr BP. This period of more intensive use is reflected not only in an increase in chipped stone artefacts and charcoal but also in the concentration of grindstones, and emu egg-shell in these levels.

Distinctive late Holocene artefacts, such as backed blades and tula adzes, first appear in the middle levels of layer I (18 cm), estimated to date from about 3,000 yr BP.

The significance of this site must be seen in the context of the human colonization of the Australian arid zone. This region is presumed to have been the most difficult of any Australian environment encountered by Pleistocene immigrants from the Indo-Malaysian region and its settlement therefore provides some indication of the adaptability of these early human groups. It has been postulated that initial settlement of the desert was primarily controlled by the ability of the original immigrants to adapt to new plant resources, especially seeds^{9,10}, or by the time required to modify an existing subsistence pattern narrowly focused upon littoral, lacustral and riverine resources¹. The accumulation of evidence from Puritjarra, and other sites in the Pilbara^{11,12}, Flinders ranges⁵, Strzelecki dunefield¹³, and elsewhere¹⁴, now indicates that the arid zone was widely settled by 13–15,000 yr BP and that some desert uplands were occupied before 20,000 yr BP. Settlement of the continent in the immediate landfall era may well have concentrated upon northern and eastern Australia⁹, regions with a broad suite of Indo-Malaysian plant food species and extensive riverine resources, but the penetration of the desert now appears to have been completed much earlier than previously thought and to have been independent of the development of distinctive seedgrinding technology¹⁵.

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Proto-oncogene *c-fos* expression in growth regions of fetal bone and mesodermal web tissue

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The phylogenic conservation of the proto-oncogene *c-fos* suggests that this gene product is required for normal metabolic processes. Investigations into the transcription pattern of *c-fos* in normal tissues and cells^{1–8} have revealed expression during development, differentiation and growth^{1,3–7} which is dependent to a large extent on external signals transferred by growth factors^{9–15}. The complex pattern of stage and tissue-specific expression has raised the hypothesis that the *c-fos* gene product might function in the control of either proliferation or differentiation^{7,16}. However, no detailed analysis is yet available concerning the normal expression of *c-fos* during embryonic and fetal development. Interestingly, recent data derived from studies in transgenic mice reveal that the biological effect of overexpression of exogenous *fos* is restricted to the developing bone tissue and T-cell development of the mice, perhaps signifying that these cells represent a physiological target tissue of the proto-oncogene *fos*¹⁷. Thus, to gain deeper insight into the functional role of the *c-fos* gene product during physiological processes, it is a requirement to carry out a detailed analysis of the localization and cell-type specificity of *c-fos* expression in normal mouse embryos. Using the technique of *in situ* hybridization, we demonstrate here that stage-specific expression of the proto-oncogene *c-fos* in mouse embryos is restricted to the perichondrial growth regions of the cartilaginous skeleton. Moreover, we found strong *c-fos* transcription in web-forming mesodermal cells, which are also characterized by a stage-specific high growth capacity. Our results suggest a tissue-specific regulatory role of *c-fos* during differentiation-dependent growth processes of fetal bone and mesodermal web tissue.

In order to analyse the normal expression pattern of *c-fos* in embryonic tissues on the cellular level, we performed *in situ*

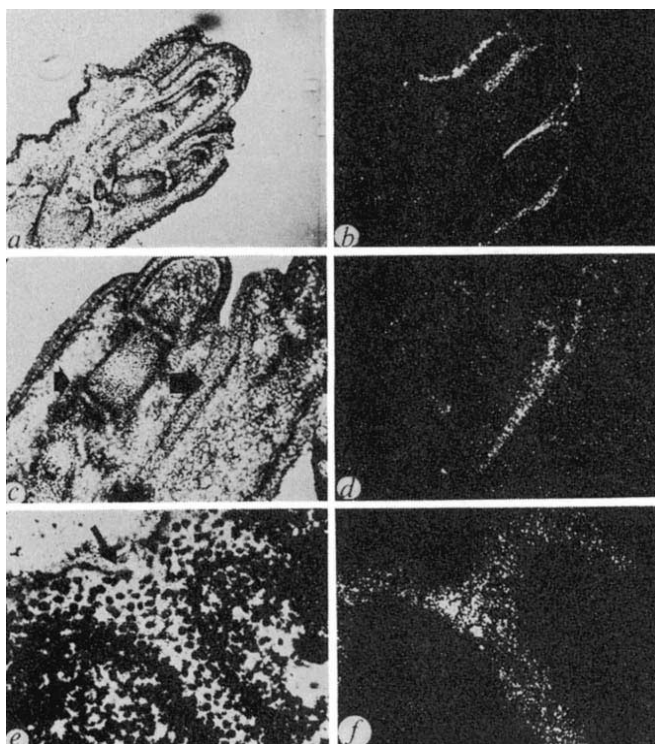


Fig. 1 Expression of the proto-oncogene *c-fos* in sections of the fore- and hindfoot of 17-day-old mouse embryos. *a, b*, Bright- and dark-field photograph of a frontal section of the forefoot. *c, d*, Bright- and dark-field photographs representing toes of the hindfoot (the large arrow indicates the localization of the web tissue, the small arrow that of the blastemal interzones). *e, f*, Higher magnification of the web tissue joining the toes of the hindfoot. The arrow indicates the localization of the outer epidermis.

Methods. Embedding, sectioning and fixation of the embryos was performed as described previously²⁶. SP6-labelled RNA probes were prepared using a 1 kilobase (*Pst*I-*Pst*I) fragment of *c-fos* complementary DNA²⁷ and a murine homoeo box-containing clone Hox 1.3 (*Eco*RV, *Eco*RI) (M. Fibi, B. Zink, M. Kessel, A. M. Colberg-Poley & P. G., manuscript submitted) cloned into the polylinker region of PSPT18 vectors (Promega Biotec) by standard methods. High specific activity RNA (approximately 1×10^8 c.p.m. μg^{-1}) was prepared from the coding and anticoding strand of the fragments using 100 μCi [α -³⁵S] UTP (400 Ci mol^{-1} , Amersham). After removal of unincorporated labelled nucleotides, probes for *in situ* hybridization were subjected to limited alkaline hydrolysis to generate a size range of about 50–100 bp²⁸. Probes were stored in solution with 50% formamide and 10 mM dithiothreitol at -20°C . Section pretreatment was performed as described^{26,29}. After incubation in $2 \times \text{SSC}$ (30 min, 70°C) to remove basic proteins, sections were treated with pronase for 10 min at room temperature. Slides were washed in PBS, fixed again in 4% paraformaldehyde, acetylated, dehydrated and air dried. Hybridization and washing were carried out as described by Ingham *et al.*³⁰. For autoradiography the slides were immersed in Kodak NTB 2 emulsion (diluted 1:1 with water), air-dried and autoradiographed for ~7 days at 4°C in a dry, light-tight box. Slides were developed at 15°C in Kodak D19 developer for 3 min, rinsed briefly in a water stop bath and fixed for 10 min in Kodak Unifix. Finally, slides were stained with Giemsa for light microscopy.

hybridizations to thin sections of mouse embryos at different stages of development. The antisense strand of *c-fos* and a heterologous DNA were transcribed and used for parallel hybridization as controls. In no case could we detect non-specific hybridization with these controls, except in embryonic membranes where hybridization with labelled sense RNA revealed a detectable signal (data not shown). Further control hybridiz-

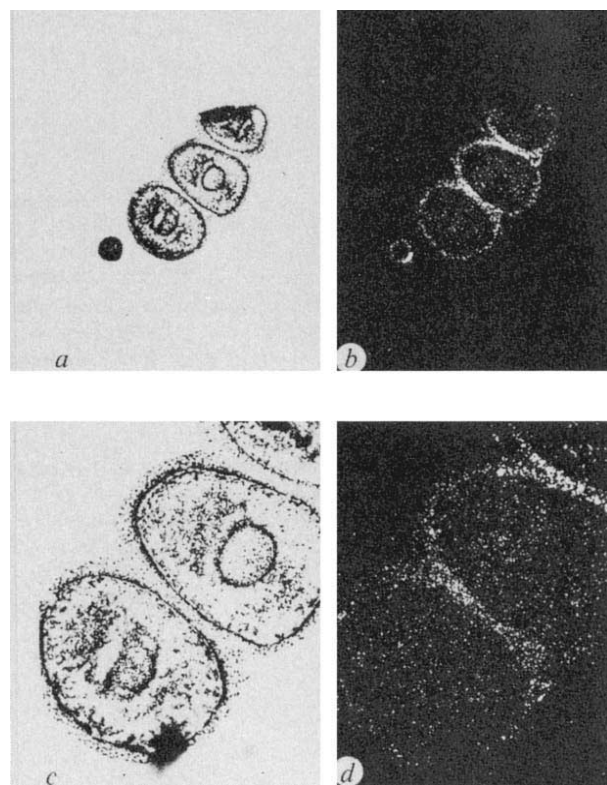


Fig. 2 Localization of *c-fos*-specific RNA by *in situ* hybridization on a transverse section of the fingers from a 17-day-old mouse embryo. *a, c*, Bright field photographs from the lower and higher magnification of the Giemsa stained section. *b, d*, Corresponding dark field photographs revealing the distribution of the label. *In situ* hybridization was carried out as described in Fig. 1. Autoradiography was for 7 days.

ations to sections of mouse placenta (13 day) were performed to establish the degree of specificity of the method. It has already been demonstrated¹⁸ by RNA dot blot analysis that *fes*-specific transcripts are located in outer portions of the mid-gestation placenta. Our *in situ* hybridization experiments also showed expression of *fes*-specific transcripts in the outer spongiotrophoblast of the 13 day murine placenta (data not shown).

Having confirmed the specificity of the signal, we examined the expression of *c-fos* in mouse embryos. Figure 1 shows several frontal sections of the fore- and hindfoot regions of 17-day-old mouse embryos (Fig. 1*a, c, e*). At this stage of development the fingers and toes are just becoming parallel and are joined by newly generated web tissue^{19,20}, indicated by the arrow in Fig. 1*c*. *In situ* hybridization of the sections using a *fes*-specific probe reveals expression restricted to the subcutaneous connective web tissue of the fore- and hindfoot (Fig. 1*b, d, f*). A strong spatial restriction of *fes* expression to the web-forming cells is evident, whereas the region of the outer epidermis (indicated by the arrow) is completely negative. *In situ* hybridization to transverse sections of the hindfoot from another 17-day-old mouse embryo confirm these results (Fig. 2*a-d*). Again, a dense accumulation of grains can be seen in the connecting web tissue of the toes (Fig. 2*b-d*). Interestingly, this tissue, which is characterized by high levels of *fes* RNA, is the site of major changes in the orientation of fingers and toes at this stage of development. Whereas in 15-day-old mouse embryos the toes and fingers are still separated, during the onset of embryogenesis up until day 18 the webbing of toes and fingers is mediated by a rapid outgrowth of mesodermal cells, giving rise to the webs of the

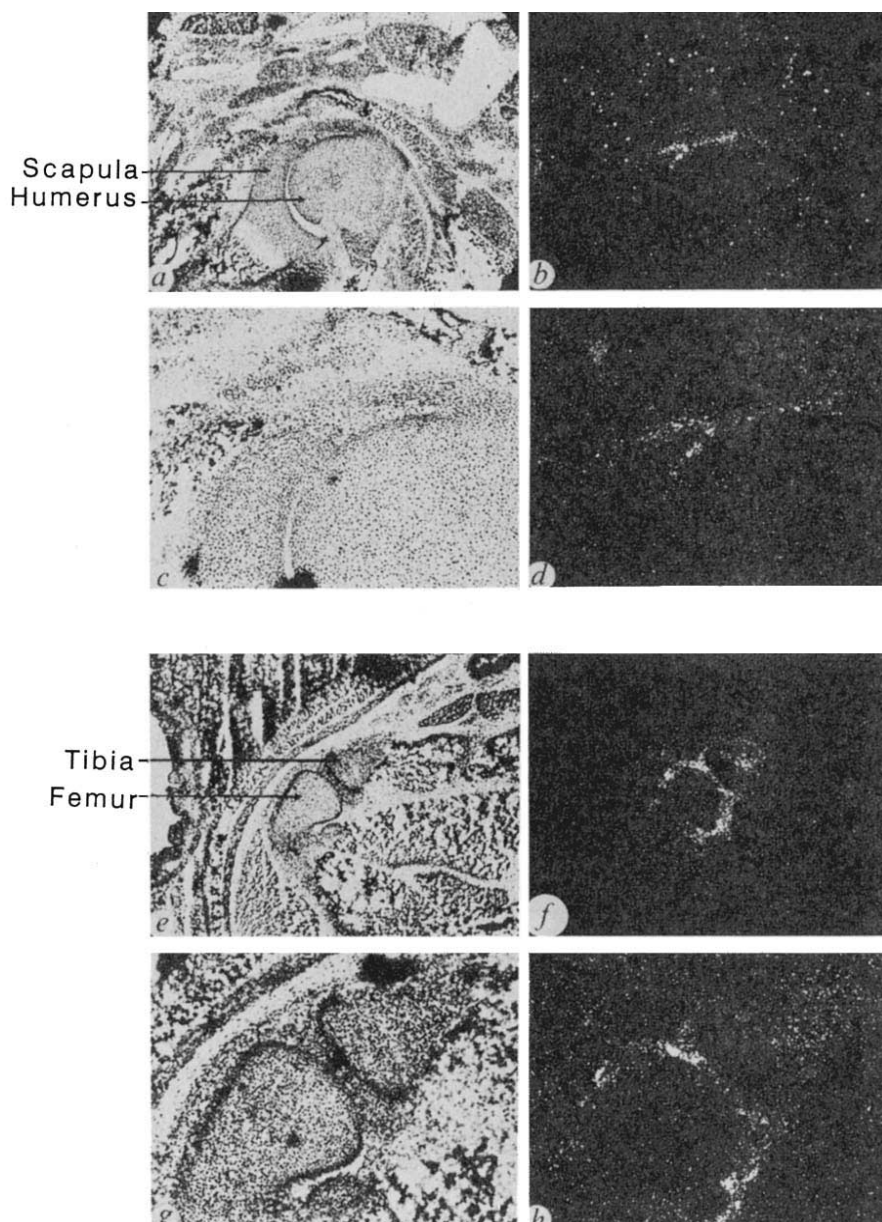


Fig. 3 *In situ* hybridization to sagittal sections from embryonic long bones (day 17) using a *fos*-specific RNA probe. *a-d*, Bright and dark field photograph of the joint region of humerus and scapula (lower and higher magnification of the same section). *e-h*, Joint region of the long bones tibia and femur (bright and dark field photographs are shown in different magnifications). *In situ* hybridization was performed as described in Fig. 1, except that exposure time was about 2 weeks.

newborn mouse^{19,20}. Thus, the pronounced expression of *c-fos* in this tissue, which is typified by a very high growth rate, might indicate a regulatory role of *c-fos* gene product in the normal differentiation-specific outgrowth of this tissue. It can be seen (Fig. 1*c* and *d*) that, besides the strong *fos*-specific signal of the webs, a clear accumulation of grains is also located in the blastemal interzones of the toe cartilage (Fig. 1*c*), stimulating us to analyse the expression of the *c-fos* gene in embryonic bone in more detail. At day 17 of development all bones are well chondrified and ossification of most of them is in advanced progress¹⁹. Moreover, the long and tubular bones in particular are characterized by an enhanced growth rate. Their cartilage growth consists mainly of addition of new cells at the ends (apposition) and in the tissue forming the interzones of the joints²¹.

Figure 3 (*a-h*) shows several sections of long bone tissue with the corresponding joints of femur, tibia, humerus and scapula. *In situ* hybridization to these sections gives a clear signal of *fos*-specific RNA in the perichondrium enclosing the end of the bones which is where the youngest cells are located and most growth takes place²¹. Expression of *fos*-specific RNA is also detectable in the blastemal interzones of the embryonic

knee and shoulder joints (Fig. 3*b, d, f, h*).

Comparable results were obtained from *in situ* hybridization to sections of the tarsal region of the embryo. Figure 4 demonstrates the location of talus, calcaneus, navicular and cuneiforme, which belong to the class of short, irregular bones²¹. Of these tarsal elements, only the talus and the calcaneus begin to ossify prenatally, whereas the others do not ossify until after birth. One of the main changes, especially of the talus, is an increase in width and height relative to length. This bone is distinguished by more extensive changes during the fetal period compared with the other tarsal bones²¹. Expression of *fos*-specific RNA occurs significantly in the lateral flanking regions of the talus, where most of the growth takes place (Fig. 4*b*). Finally, to see whether embryonic bone or bone precursors (such as the bone-forming mesodermal condensations) at earlier stages of development also show the expression of *fos*-specific RNA, we performed *in situ* hybridization to serial sections of 13-day and 10-day-old mouse embryos. No expression could be detected either in presumptive bone or in other tissues of these embryos (data not shown).

Our *in situ* hybridization experiments to investigate the physiological expression pattern of the *fos* gene in mouse embryos

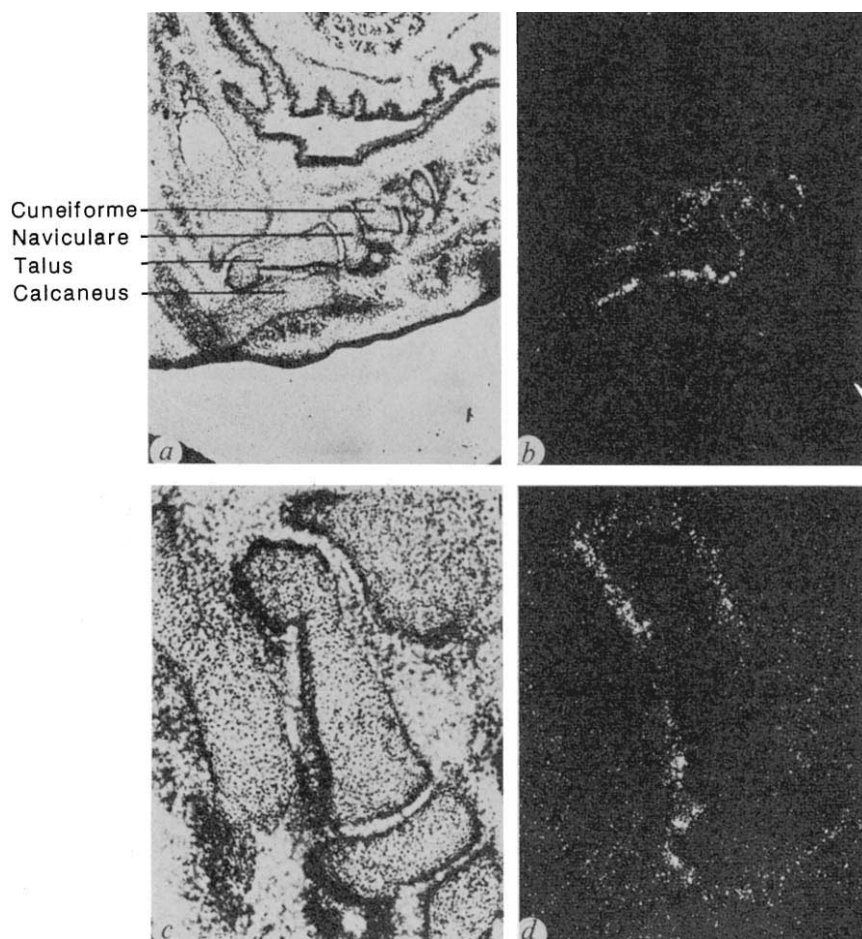


Fig. 4 Localization of *c-fos*-specific RNA by *in situ* hybridization on sections of the tarsal region of a 17-day-old mouse embryo. *a, b*, Bright and dark field photograph of the tarsal region containing small irregular bones, such as cuneiforme, naviculare, talus and a smaller portion of the calcaneus.

suggest that the *fos* gene product probably functions at a time in embryogenesis when the cartilaginous skeleton has already been constructed. The restriction of *c-fos* RNA expression to those parts of fetal bone having the highest growth potential at about day 17 of development suggests that the *fos* gene product might have a regulatory function in these tissue and stage-specific growth processes. The involvement of *c-fos* proto-oncogene in growth processes has been studied in detail⁸⁻¹⁵. Recent analysis of the *c-fos* promoter region has demonstrated an element which is essential for inducibility in response to serum growth factors²²⁻²⁴. Moreover, the rapid induction of *c-fos* in cell culture in response to mitogens⁸⁻¹⁵, together with the finding that the expression of the gene in primary amnion cells is regulated by placenta- and embryo-derived factors, has raised

the possibility that regulation of the *c-fos* proto-oncogene by external signals may be a general phenomenon¹⁵.

The results we present here, together with data recently obtained in transgenic mice¹⁷, indicate that developing bone cells probably represent a normal target tissue of the proto-oncogene *fos* in fetal and newborn mice. The controlled growth of embryonic bone might also be the result of growth factor functions, resulting in a balance between growth, bone resorption and remodelling^{17,25}. Thus, the physiological expression of the *c-fos* gene in the developing bone tissue might be a consequence of these interactions.

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Possible positive autocrine feedback in the prereplicative phase of human fibroblasts

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The growth of normal diploid fibroblasts is generally thought to be tightly controlled by exogenous growth factors such as platelet-derived growth factor (PDGF) and epidermal growth factor (EGF). Subversion of a growth factor pathway at a regulatory point is considered to be a key event in neoplastic transformation and tumorigenesis¹. Thus, simian sarcoma virus has acquired the gene encoding the B-chain of PDGF²⁻⁴ and there is direct experimental proof that SSV-transformation is mediated by a PDGF-like growth factor⁵. There is accumulating evidence that PDGF-like molecules are also synthesized and released by certain normal cells⁶⁻¹³, suggesting an important role of cellularly produced PDGF in development and tissue regeneration. We now present evidence that a transient expression of the gene encoding the PDGF A-chain, and the synthesis and release of functional A-chain homodimers, is an early event in the prereplicative phase of normal human foreskin fibroblasts exposed to PDGF or EGF. Since these cells are PDGF-responsive, the results imply the existence of a positive autocrine signal that may serve as an amplifier of the mitogenic response under certain conditions.

Human diploid foreskin fibroblasts were rendered quiescent by serum starvation for four days. Following the addition of EGF or PDGF, the levels of PDGF A-chain and B-chain (*c-sis*) messenger RNA were determined by Northern blot hybridization using ³²P-labelled cDNA probes. As seen in Fig. 1, addition of either mitogen caused a transient expression of PDGF A-chain mRNA; transcripts of the expected sizes, 1, 9, 2.3 and 2.8 kilobases (kb)¹⁴ were detected after two and four hours of stimulation but undetected in control cultures and in growth factor treated cultures at later time points. Hybridization with the PDGF B-chain (*c-sis*) probe did not yield any detectable signal (not shown).

To determine whether stimulation of PDGF A-chain mRNA requires *de novo* protein synthesis, the effect of cycloheximide was analysed. As seen in Fig. 2, the addition of cycloheximide had a slight stimulatory effect on its own, which was additive to the effect of PDGF. Thus, PDGF-stimulation of A-chain mRNA expression is a direct effect involving post-translational modification rather than synthesis of novel proteins. The time-dependence of PDGF A-chain expression was transient even in the presence of cycloheximide; eight hours after stimulation the expression had returned to the base level (Fig. 2).

We next searched for protein product(s) of the induced PDGF A-chain gene by immunoprecipitation of ³⁵S-cysteine-labelled cultures using an antiserum directed against native PDGF¹⁵ or antisera raised against a synthetic peptide; the latter specifically recognizes the A-chain of reduced PDGF (A.H. *et al.*, unpublished data). ³⁵S-cysteine labelling was performed during a 2-5 hour interval after addition of EGF followed by a 1.5 hour chase period. Unstimulated cultures were used as controls. The anti-PDGF serum precipitated components of relative molecular mass (*M_r*) 31,000 (31K) and 37K from the culture medium of EGF-treated cultures (Fig. 3a). Upon reduction and alkylation, species of 23K and a minor, slightly faster migrating component were obtained, as well as two closely migrating species of *M_r* 16,500 and 17,000. Direct evidence that these components are related to PDGF A-chains was obtained by immunoprecipitation

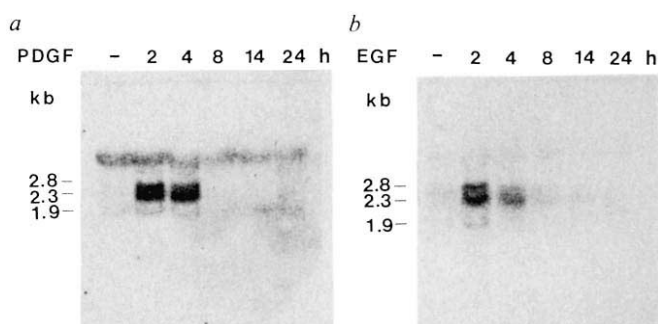


Fig. 1 Effect of *a*, PDGF and *b*, EGF on the levels of PDGF A-chain mRNA in human diploid neonatal foreskin fibroblasts, line AG 1523, as determined by Northern blot hybridization analysis. AG 1523 cells were rendered stationary by a 4-day incubation in serum-free MCDB 104 medium with a reduced calcium concentration (0.5 mM). The cells were stimulated with 10 ng ml⁻¹ PDGF or 10 ng ml⁻¹ EGF for different time periods. Total RNA was isolated using the LiCl/urea method¹⁸. The RNA samples (15 µg) were separated on 1% agarose gel containing 2.2 M formaldehyde blotted onto a nitrocellulose filter (Schleicher and Schüll) and hybridized to a ³²P-labelled PDGF A-chain cDNA probe¹⁴. The hybridization time was 40-48 h at 42°C in hybridization mixture containing 50% formamide, 5× SSC, 5 mM EDTA, 0.5% SDS, 1× Denhardt's and 250 µg ml⁻¹ denatured salmon sperm DNA. The filters were washed in 0.1× SSC, 0.5% SDS for 5-6 h, dried and exposed at -70°C to Kodak XAR-5 film for 14 days.

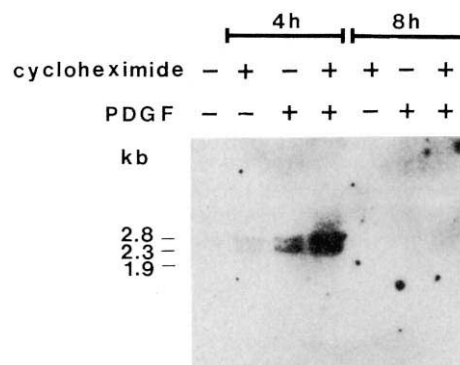


Fig. 2 Effect of cycloheximide on PDGF A-chain mRNA levels in human fibroblasts as determined by Northern blot hybridization analysis. Serum-starved cells were treated for 4 or 8 h with cycloheximide (10 µg ml⁻¹) and PDGF (10 ng ml⁻¹) as indicated. Extraction of RNA and Northern blot hybridization were performed as described in the legend to Fig. 1.

with the A-chain-specific peptide antibodies as a 16.5/17K doublet was specifically precipitated from reduced and alkylated medium (Fig. 3b); upon prolonged exposure a 23K component could also be visualized (not shown). The major cellular component recognized by the A-chain peptide antibodies was a 23K protein (Fig. 2b). In view of the predicted size of the primary translational product of the PDGF A-chain¹⁴, this component may be a precursor peptide. Consequently, the unreduced 31K component is probably the mature product being a PDGF A-chain homodimer, similar to the factor previously isolated from a human osteosarcoma cell line¹⁶.

If EGF-stimulated cells release a functional PDGF A-chain dimer, such a factor should display functional properties, for example compete with ¹²⁵I-labelled PDGF for binding to the PDGF receptor, or cause a blockade and receptor down-regulation of the producing cells. Figure 4 shows an accumulation of PDGF receptor-competing activity in the conditioned medium

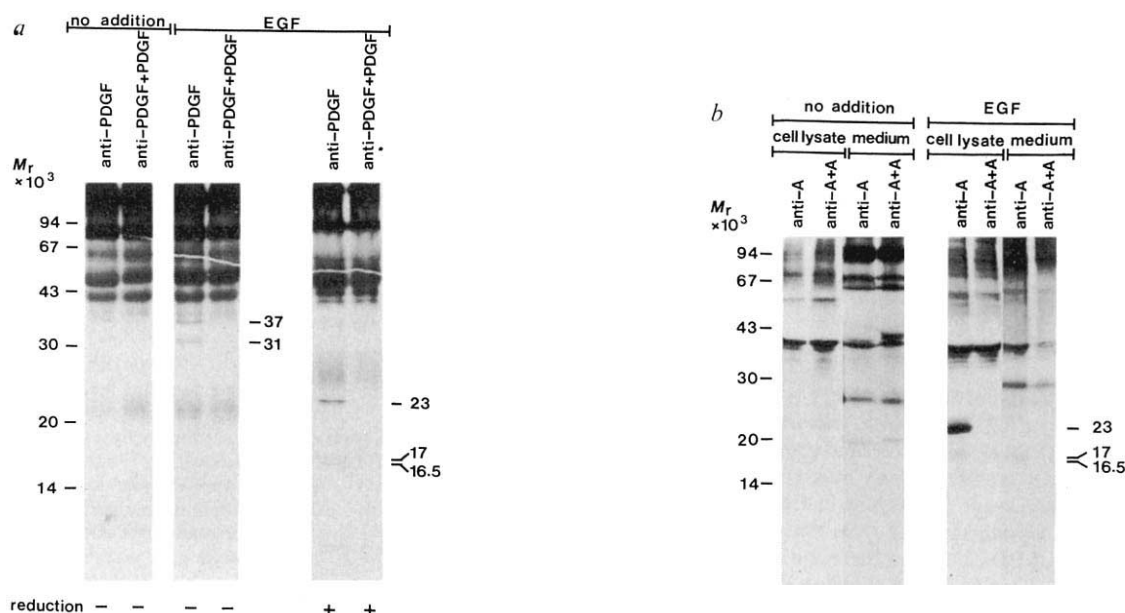


Fig. 3 Immunoprecipitation of PDGF-like proteins from EGF-stimulated foreskin fibroblasts using an antiserum directed against *a*, native PDGF or *b*, a peptide antiserum directed against the PDGF A-chain. Serum-starved foreskin fibroblasts in 150 cm² culture flasks were preincubated with EGF (10 ng ml⁻¹) for 2 h. In the presence of EGF the cells were then labelled for 3 h with ³⁵S-cysteine (Amersham 600 mCi mmol⁻¹) in 2 ml cysteine-free MCDB 104 medium per flask and chased for 1.5 h with 2 ml of medium. Phenyl methyl sulphonyl fluoride (PMSF) (0.5%), aprotinin (Trasylol) (2.5%) and Triton X-100 (0.01%) (final concentrations) were added to the pooled pulse and chase media. The cells were lysed by freeze-thawing in the presence of 0.5 M NaCl, 10 mM phosphate buffer pH 7.4 and PMSF (15%), aprotinin (7.5%) and Triton X-100 (0.03%), and diluted 3 times in 10 mM phosphate buffer. In *a*, conditioned media were incubated overnight at 4 °C with 50 µl normal rabbit serum. Packed protein A Sepharose (Pharmacia) beads were added (100 µl) and incubated for 2 h. After centrifugation, 50 µl of PDGF antiserum, or the same amount of antiserum blocked with 1 µg of PDGF, were added to the supernatants. After incubation overnight at 4 °C, 100 µl of Sepharose beads were added and incubated for 2 h. The beads were washed 4 times with 0.5 M NaCl, 10 mM Tris Buffer, pH 7.4, 0.1% Tween 80, 5 mg ml⁻¹ BSA and finally with 10 mM Tris buffer pH 7.4. Immunocomplexes were extracted with 100 µl of 3.6% SDS, 80 mM Tris pH 8.8, 0.01% bromophenol blue and heated at 95 °C for 3 min. One aliquot of the supernatant was reduced by incubation with 10 mM DDT for 3 min at 95 °C and alkylated with 50 mM iodoacetamide. The other part was kept non-reduced. All samples were analysed by SDS-gel electrophoresis¹⁹ using gradient gels (13–18% acrylamide) and fluorography. In *b*, conditioned media and cell lysates were treated with 10 mM DDT (2 h, 37 °C) followed by 50 mM iodoacetamide (0.5 h at room temperature). After pH adjustment, the material was immunoprecipitated as above using a rabbit antiserum against a synthetic peptide specifically recognizing the PDGF A-chain (Hammacher *et al.*, unpublished data). Samples were analysed by SDS-gel electrophoresis and fluorography as above.

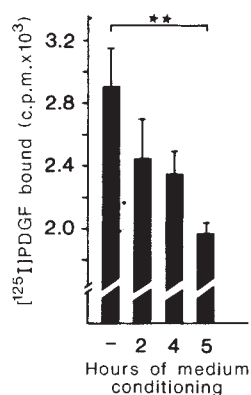


Fig. 4 PDGF receptor-competing activity of conditioned medium from EGF-stimulated human foreskin fibroblasts. Subconfluent cultures of human fibroblasts in 150 cm² culture flasks were incubated for 4 days in serum-free MCDB medium. Medium containing 10 ng ml⁻¹ of EGF (1 ml per culture) was added and incubated on a rocket platform. Media were harvested at intervals and analysed for PDGF receptor-competing activity as described²⁰. Five cultures were used for each time point, and the experiment was repeated three times. Values are given as mean ± s.e.m. and analysed by the Student's *t*-test. ***P* < 0.02 compared with the untreated cell culture.

of quiescent, serum-starved fibroblasts after EGF addition (10 ng ml⁻¹). The PDGF receptor competing activity was equivalent to about 3 ng of PDGF per ml. These results provide compelling evidence that the addition of EGF (or PDGF) to quiescent human diploid fibroblasts causes a transient expression of the PDGF A-chain mRNA and synthesis of a functional protein.

Recent findings have implicated the existence of a negative feedback loop, linked to growth factor-induced mitogenesis and executed by released growth inhibitory proteins. Thus, the addition of PDGF to mouse 3T3 cells¹⁷ leads to the expression of interferon-β mRNA. Moreover, exposure of human fibroblasts to PDGF leads to an expression of transforming growth factor-β (TGF-β) mRNA (Y.P. *et al.*, unpublished data), TGF-β being a growth inhibitor for a variety of cell types including human fibroblasts. The functional significance of the transient PDGF A-chain expression in growth stimulated cells is at present not known but we would like to suggest that it may reflect the presence of a positive regulatory mechanism in the prereplicative phase, that could serve as an amplifier of the initial growth stimulus. The ultimate growth response to a given mitogenic stimulus may thus be modulated by autocrine mechanisms involving both negative and positive feedback loops.

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Evidence for local stimulation of ACTH secretion by corticotropin-releasing factor in human placenta

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The hypothalamic-pituitary-adrenocortical axis is activated in pregnancy and parturition^{1–4}. Levels of immunoreactive corticotrophin releasing factor (irCRF), immunoreactive adrenocorticotrophic hormone (irACTH) and cortisol concentrations in maternal plasma are elevated throughout gestation, increase further during labour and fall precipitously after parturition^{5,6}. The placenta contains biologically active CRF and ACTH and it has been suggested that the placenta produces these peptides during pregnancy^{7–10}. Here we show that irCRF is located in the cytotrophoblast cells of placenta collected at term. Using a monolayer primary culture of human placental cells we have found that CRF stimulates secretion of peptides containing the ACTH sequence in the placenta in a dose-dependent manner, as it does in the pituitary. This effect is reversed by a CRF antagonist and is mimicked by dibutyryl cyclic AMP and forskolin. Glucocorticoids, which suppress the secretion of pituitary ACTH, were found to have no influence on release of irACTH by the placenta. Oxytocin and prostaglandins stimulate irACTH and irCRF secretion from cultured placental cells and the irACTH-releasing activity of two prostaglandins is partially reversed by a CRF antagonist. Thus CRF may be involved in the paracrine regulation of placental irACTH secretion.

Indirect immunofluorescence staining of immersion-fixed human placenta collected at term revealed numerous cells stained positively for human irCRF (Fig. 1). These were concentrated overwhelmingly in the cytotrophoblast layer of placental villi, with no clear indication of widespread staining of syncytiotrophoblasts. We cannot, however, exclude the possibility that a relatively small fraction of syncytiotrophoblasts might express CRF-like immunoreactivity. This is the first reported immunohistochemical localization of a CRF-like peptide in placenta, but previous studies had reported the presence of a number of hypothalamic and pituitary hormones in placental trophoblast tissue^{11–13}. The majority of pituitary hormones, including ACTH, were localized primarily within the

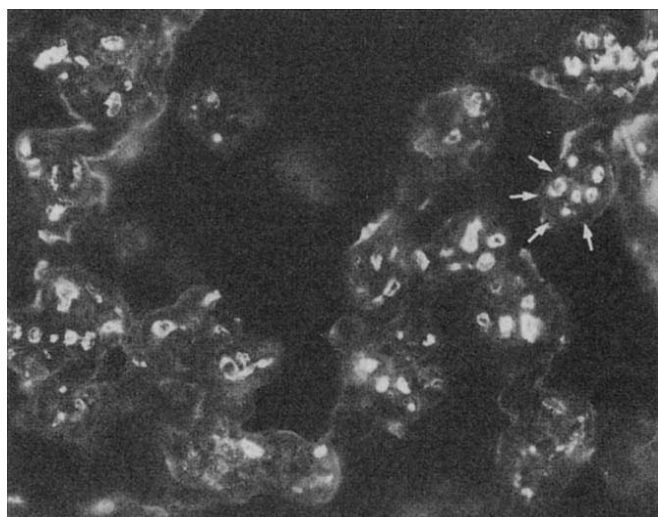


Fig. 1 Fluorescence photomicrograph of human placenta showing CRF-like immunoreactive cells in the cytotrophoblast layer of the placental villi. Cells in the syncytiotrophoblast layer (arrows), at the periphery of the villi, are unstained. Magnification $\times 210$.

Methods. Fresh term placenta was fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer for 4–6 h at room temperature, and stored overnight at 4°C in the same fixative with 10% sucrose added. Frozen sections 20- μ m thick were cut in cryostat and were incubated for 48 h at 4°C in a 1:2,000 dilution of a polyclonal antiserum against human CRF (serum C70)²⁸. General staining procedures and buffers are described elsewhere²⁹. Primary antisera were localized using fluorescein-conjugated goat anti-rabbit IgG (Tago); 5% nonfat dry milk was added to minimize nonspecific binding of the secondary antibody³⁰. No specific staining was observed when preimmune serum was substituted for anti-CRF, or after preincubation of the primary antiserum overnight at 4°C with 100 μ g ml⁻¹ synthetic human CRF.

syncytiotrophoblast layer, while gonadotropin-releasing hormone (GnRH) and somatostatin have been described in cytotrophoblasts.

The addition of CRF to primary trophoblast cell cultures stimulated irACTH secretion in a dose-dependent manner (Fig. 2a). The amounts of irACTH were measured by radioimmunoassay (RIA) using an antiserum provided by Dr D. Orth¹⁴ that is directed against the ACTH (11–24) sequence. Although this RIA does not distinguish between ACTH and its intact or partially processed precursors, the placenta is reported to contain biologically active ACTH and the majority of irACTH in acidic placental extracts elutes in the size range of ACTH during gel filtration chromatography¹⁰. The concentration of CRF required for 50% of maximal stimulation (EC_{50}) of irACTH secretion was 35.5 $\pm$ 7.5 nM (mean $\pm$ s.e.m. of four experiments) which is higher than the EC_{50} of CRF to release irACTH from cultured anterior pituitary cells or of β -endorphin from cultured neurointermediate lobe cells¹⁵. The CRF antagonist, rCRF (12–41) (1 μ M), synthesized as previously described¹⁶, reversed the stimulatory action of CRF on irACTH secretion from placental cells, supporting the notion of a specific action of the releasing factor (Fig. 2a). Furthermore, several other neurohormones, including GnRH, thyrotropin-releasing hormone (TRH) or growth hormone-releasing factor (GRF) had no effect on irACTH release (data not shown). A further support to the specificity of this action is the evidence that CRF binding sites seem to be present on cultured placental cells (M. H. Perrin, F.P. and W.V., manuscript in preparation). Dibutyryl cyclic AMP and forskolin, a diterpene that stimulates adenylate cyclase activity, both significantly increased secretion of irACTH secretion from placental cells (EC_{50} of 50–200 μ M) (Fig. 2); the intrinsic activity (secretory response at maximally effective con-

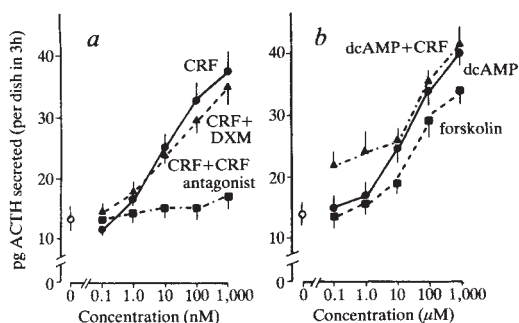


Fig. 2 Effect of CRF with and without: *a*, dexamethasone, (DXM; 10 μ M) or CRF antagonist (1 μ M), or *b*, dibutyryl cyclic AMP or forskolin on ACTH secretion from placental cell cultures. Each point represents the mean $\pm$ s.e.m. Human placentae collected from women undergoing elective caesarean sections at term pregnancy were used to prepare the trophoblast cultures. Chunks of tissue were minced and rinsed three times before dissection of the connective tissue. Cells were dispersed both enzymatically (0.4% collagenase II, type II, Cooper Biomedical and 3,000 KU DNase type IV, Sigma in HEPES dissociated buffer, HDB) and mechanically in a water-jacketed spinner suspension flask (Wheaton Scientific). After 1 h trypsin (0.4%, 85,800 BAEE Units ml^{-1} , Sigma) was added and the solution was stirred for 10 min more. The dissociation mixture was filtered through a 42- μ m filter (Spectrum Med.) and centrifuged twice at 300 g and 400 g for 10 min at room temperature. Pelleted cells were resuspended in culture medium (β -PJ added with 10% fetal calf serum, HyClone Laboratories, and Sato's cocktail²⁸) and in 0.1% bovine serum albumin (BSA; Pentex, Miles Laboratories) HDB solution and centrifuged at 300 g for 10 min. The unfiltered cells were further stirred for 10 min in the suspension flask and successively subjected to the above procedure. Cells obtained from each digestion were resuspended in culture medium and plated in 35-mm six-well multi-dishes (Costar) at 2.5 ml culture medium per well, previously coated with poly-D-lysine (Sigma). The entire procedure yielded 3×10^6 cells per g of tissue and 5.0 – 6.5×10^5 cells per well ($98 \pm 2\%$ of viability by trypan blue exclusion). The cultures were maintained at 37 $^{\circ}\text{C}$ in a water saturated atmosphere containing 5% CO_2 , changing the culture medium every 2 days. The experiments were done one week after plating. On the day of the experiment the cells were washed twice with HEPES buffered Krebs' Ringer bicarbonate (HKRB) and 0.1% BSA solution, and preincubated for 1 h with the same solution. The various secretagogues, prepared as 100-fold concentrates and added in small volumes to triplicate wells, were incubated for 3 h in 500 μ l HKRB containing bacitracin ($60 \mu\text{g ml}^{-1}$) (Sigma) and ascorbic acid ($50 \mu\text{g ml}^{-1}$; Sigma). Vehicle-treated wells (control) were present in each assay. At the end of the incubation the conditioned media were collected and ACTH concentrations were measured by radioimmunoassay (RIA). Porcine ACTH(1–39) was used to prepare the standard curve and for iodination (chloramine-T method) to produce the tracer (purified by high performance liquid chromatography, HPLC). Ovine anti-human ACTH serum, provided by Dr D. Orth¹⁴, was used at the final dilution of 1:250,000. All reagents were diluted in 0.1% BSA 0.1% Triton X-100 saline phosphate buffer (pH 7.4). After 24 h incubation at 4 $^{\circ}\text{C}$, rabbit anti-ovine α -globulin (1:10 dilution) was added for a further 18 h. Separation of bound from free tracer was obtained by centrifuging at 2,300 g for 45 min, after washing with 1.0 ml of 2.5% fraction V BSA saline phosphate buffer (pH 7.3). Assay sensitivity was 2.0 pg/tube, with IC_{50} 30 pg. This RIA, which recognizes amino acids 11–24 of ACTH, does not react with α -MSH, β -MSH or β -LPH but detects placental POMC derived proteins containing the ACTH sequence¹⁴. Inter- and intra-assay coefficients of variations were 7% and 4.0% respectively. Nonspecific RIA interference was controlled in samples containing the drugs under the same conditions as the culture samples assayed. Recovery of standard ACTH exogenously added to the cultures (100 pg/well) was $90 \pm 3\%$. CRF(12–41) was synthesized using the solid phase approach¹⁶. After HPLC purification the preparation ($>98\%$ pure) was characterized thus: $[\alpha]_D = -23.5^{\circ}$ C = 1 in 50% acetic acid; amino acid analysis after acid hydrolysis gave the expected integer values. Isocratic conditions on HPLC: 0.1% TFA in 36.6% acetonitrile/ H_2O , retention vol. 10.1 ml; column from Vydac (0.46 $\times$ 25 cm), 5 μC_{18} .

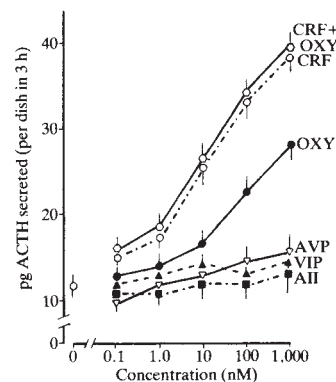


Fig. 3 Effect of oxytocin, [Arg⁸]vasopressin (AVP), VIP and angiotensin II (AII) on ACTH secretion and on CRF-induced ACTH increase in placental cell cultures.

centrations) of dibutyryl cAMP was equal to that of CRF. Immunoreactive ACTH release induced by CRF (10 nM) was not potentiated by dibutyryl cAMP (Fig. 2b). These observations lead to the suggestion that cyclic AMP serves as intracellular mediator of the action of CRF on irACTH secretion in human placenta. The adenylate cyclase/cAMP system is present in human placenta^{17,18} and it participates in the regulation of secretion of various placental hormones, including human chorionic gonadotropin (hCG)¹⁹ and inhibin²⁰. The same intracellular mechanism of action has been proposed to mediate the CRF stimulation of irACTH by the pituitary¹⁵. Conversely, the basal and CRF-stimulated secretion of irACTH from cultured placental cells was not modified by even very high levels of dexamethasone (10 μ M) (Fig. 2a), showing that, in contrast to pituitary cells²¹, glucocorticoids do not modulate irACTH secretion from cultured human placental cells. It has been observed previously that plasma ACTH concentrations do not change in pregnant women following therapeutic administration of dexamethasone²². Our results indicate that there is no feedback inhibition of glucocorticoids on irACTH secretion from placental cells, which is indirectly consistent with the notion that this organ may be at least in part the source of maternal circulating ACTH during pregnancy.

It is known that pituitary ACTH secretion can also be modulated by vasopressin, oxytocin, VIP and angiotensin II, and these neurohormones interact with CRF to regulate basal and stress-induced ACTH secretion^{21,23}. The addition of oxytocin to placental cell cultures stimulated irACTH secretion (EC_{50} of 75–300 nM), whereas arginine vasopressin, VIP and angiotensin II were inactive (Fig. 3). The intrinsic activity of oxytocin was lower than that of CRF (Fig. 3). No peptide showed any synergistic effect on CRF-induced irACTH secretion (Fig. 3). Therefore, a stimulatory role of maternal or placental oxytocin on placental irACTH secretion might be suggested. It has been proposed that extracted human placental oxytocin is chemically identical to and as biologically active as neurohypophysial oxytocin²⁴.

Prostaglandins (PGs) are proposed to participate in the control of placental hormonogenesis. They are produced by the placenta²⁵ and increase adenylate cyclase activity and intracellular concentrations of cAMP in human placenta *in vitro*^{18,26}. The addition of PGE_2 (10 nM–1 μ M) and $\text{PGF}_{2\alpha}$ (100 nM–1 μ M) increased irACTH (Fig. 4a) and irCRF (Fig. 4b) secretion by placental cell cultures ($P < 0.01$ compared with control). PGD_2 and PGI_2 were inactive. The coinubation of CRF (12–41) (1 μ M) partially reversed the effect of both PGs on ACTH secretion (Fig. 4a). The evidence that a CRF antagonist partially reverses the irACTH releasing activity of PGs indicates that locally produced CRF may mediate prostaglandin stimulation of irACTH secretion. The discrepancy between the concentration of endogenous CRF required to stimulate irACTH secretion and the CRF concentrations observed in the culture

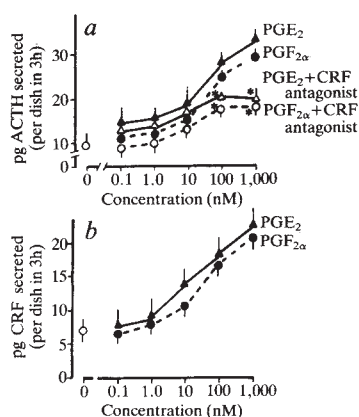


Fig. 4 Effect of prostaglandins (Sigma) on *a*, ACTH and *b*, CRF secretion from placental cell cultures. The incubation procedures followed to evaluate CRF secretion from cultured placental cells were the same as described for ACTH. At the end of the incubation, the conditioned media were collected and dried by speed-vac (Savant). The samples were redissolved in RIA buffer to measure CRF concentrations. No significant difference in CRF concentrations was found between extracted (using Bond-Elut C₁₈ cartridges, Analytichem) and non-extracted samples. CRF was eluted with 50% 2-propanol–50% triethylammonium formate, with a recovery of more than 80%. Synthetic rat CRF was used to prepare the standard curve and for iodination (chloramine-T method) to produce the tracer (purified by HPLC)²⁸. Rabbit anti-rat CRF was used at the final dilution of 1:770,000. All reagents were diluted in 0.1% BSA 0.05% Triton X-100 saline phosphate buffer (pH 7.3). After 48 h incubation at 4 °C (preincubation 24 h), sheep anti-rabbit α -globulin (dilution 1:40) and 10% polyethylene glycol solution was used to separate free from bound tracer (30 min incubation), washing with saline phosphate buffer (1 ml) and centrifuging at 2300 g for 45 min. The assay sensitivity was 2 pg per tube, with an IC₅₀ of 20 pg. The inter- and intra-assay coefficients of variation were 8% and 4%, respectively. The nonspecific interference with CRF RIAs of the various drugs or peptides tested were controlled. Recovery of standard CRF exogenously added to the cultures (100 pg per well) was 85 $\pm$ 5%.

medium following addition of PGs would appear to argue against a role for endogenously produced CRF in the PG-stimulated cultures. It is possible, however, that local gradients of secreted CRF might transiently exist within the cultures. Additionally, PGs might synergize with CRF to release irACTH.

In conjunction with the demonstration of CRF positive staining in human placenta, these findings lead us to propose that the release of placental ACTH is modulated by CRF *in situ*. This is similar to the hypothesis that the secretion of hCG is locally modulated by a placental GnRH-like peptide whose effect is mediated by specific high affinity receptors¹³. Thus, placental peptides related to their hypothalamic counterparts may be important in the control of hormonogenesis in the chorionic system^{13,27}. It is known that hCG and human placental lactogen, produced by syncytiotrophoblast cells, in addition to being released into the maternal circulation, influence the secretion of other placental hormones, without being transported by blood^{11–13}. Placental ACTH exhibits both paracrine activity by locally stimulating placental progesterone and estradiol production⁷ and endocrine effects in augmenting maternal and fetal adrenal steroidogenesis^{11,12}. From our results we also suggest that CRF, which is produced by placenta in sufficient quantities to exert endocrine effects at the level of the maternal pituitary^{3–7}, might also exert local paracrine actions on the production of placental irACTH and steroidogenesis.

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Incubation period of AIDS in patients infected via blood transfusion

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Temporal trends in the prevalence of HIV (human immunodeficiency virus) infection are uncertain because of the reluctance of most governments to embark on large-scale programmes of serological surveillance. In the absence of such data, attempts have been made to relate the number of reported cases of AIDS (acquired immune deficiency syndrome) in a defined population to the proportion of that population infected with the virus as a specified time point^{1–3}. One crucial determinant of this relationship is the probability distribution of the incubation period of the disease, with the period defined as the time interval from infection to diagnosis. Recent statistical analysis suggests a mean incubation period of 4.5 years⁴ with wide confidence limits, whereas a more heuristic study reports a mean of 15 years^{5,6}. Here we report on a new analysis which reveals age-related differences in

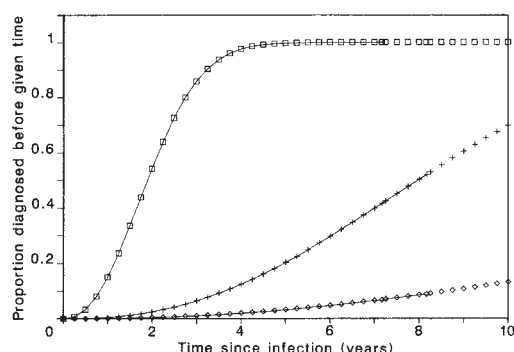


Fig. 1 The estimated incubation period described by Weibull and gamma distributions shown as the proportion of infective transfusions that are diagnosed up to a given time since transfusion. The top curve ($\square$) is the curve fitted to children (0–4 yrs old at transfusion) excluding those diagnosed after June 1985 (24 cases). It is not possible to distinguish between the fitted Weibull and gamma distributions on this scale. The middle curve (+) is the Weibull distribution fitted to adults (5–59 yrs old) diagnosed before June 1986 (126 cases). The gamma distribution fitted to the same data is shown as the bottom line ($\diamond$). The curves are shown as points to indicate extrapolation beyond existing data. The Weibull distribution has the density function $pqx^{p-1}e^{-(qx)^p}$, and the gamma $q^px^{p-1}e^{-px}/\Gamma(p)$, where p and q are parameters, and x is the time between transfusion and diagnosis. The fitted parameter values and log likelihood (l) for the Weibull distribution for children (0–4 yrs) and adults (5–59 yrs) are respectively $p = 1.939$, $q = 0.3843$, $l = 6.18$ and $p = 2.396$, $q = 0.1077$, $l = 102.24$; and for the gamma $p = 2.669$, $q = 1.098$, $l = -5.72$ and $p = 2.473$, $q = 0.0909$, $l = 102.11$. An exponential increase in incidence of infection was assumed in the above of form $a \exp(bt)$, where a and b are parameters and t is the time since the beginning of the epidemic (April 1978). The parameter values for the cases given above are $a = 0.3153$, $b = 0.7396$ (Weibull, children), $a = 13.58$, $b = 0.6404$ (Weibull, adults), $a = 0.3326$, $b = 0.7314$ (gamma, children) and $a = 83.84$, $b = 0.6431$ (gamma, adults). From these figures the expected number of adults given an infective transfusion between April 1978 and June 1986 can be calculated. It is 6,371 with the Weibull distribution and 13,796 with the gamma. This difference accounts for the difference between the middle and bottom curves (see text and Tables 1 and 2).

Table 1 Expected and observed numbers of all patients by year of transfusion and diagnosis

Year of diagnosis	1978	1979	1980	1981	1982	1983	1984	1985	1986
1978	0.01 (expected) 0 (observed)								
1979	0.19 0	0.07 0							
1980	0.56 0	0.66 0	0.14 0						
1981	0.97 0	1.69 0	1.34 0	0.28 0					
1982	1.34 0	2.81 2	3.43 2	2.71 5	0.57 1				
1983	1.58 1	3.77 2	5.70 10	6.97 7	5.50 7	1.15 3			
1984	1.67 0	4.38 5	7.64 7	11.56 15	14.13 18	11.15 20	2.33 2		
1985	1.58 1	4.54 5	8.88 8	15.49 16	23.44 25	28.66 23	22.61 26	4.73 5	
1986	0.72 0	2.19 3	4.62 3	8.80 16	14.86 13	21.49 23	24.24 14	15.04 7	0.83 1

The observed numbers have been grouped into years, although they were treated by month in the analysis. The year 1978 extends from April to December, and 1986 from January to June. The expected numbers are calculated from the model with exponential increase in incidence of infective transfusions and an incubation period following a Weibull distribution fitted to all data points. The corresponding table for a gamma distributed incubation period is very similar.

the mean (and median) incubation period: 1.97 (1.90) years for children (0–4 yrs old at infection), 8.23 (7.97) years for adults (5–59 yrs old), 5.50 (5.44) years for elderly patients (60 yrs and older).

Data from individual patients recording the times of infection and disease diagnosis are limited at present. But there is one set of data from the United States of America in which a good approximation to the time of infection has been found retrospectively in AIDS patients who received a single transfusion or short course of transfusions of infected blood or blood products^{7,8}.

Many problems surround the interpretation of these data. The number of patients infected by this route who have not yet developed AIDS is unknown, so that the data give no information whatever about the proportion of infected individuals who will ultimately develop AIDS. Individuals transfused later have been at risk of developing AIDS for a much shorter length of time so that the frequency distribution of incubation periods as directly observed is misleading. The data are subject to a number of further important caveats pointed out to us by T. A. Peterman (Centers for Disease Control, Atlanta, Georgia, USA). Namely, the report is sometimes inaccurate, incomplete or unconfirmed; there may be considerable delay between diagnosis and report; earlier cases (especially in older patients with pneumonia) may have been missed; more elderly patients may die from unrelated causes before AIDS develops, and there may be increasing efficiency of diagnosis. We have excluded all cases in which there was any doubt about dates of infection (transfusion) and subsequent diagnosis, leaving a reduced data set consisting of 297 cases out of 494 (see Table 1) transfused between April 1978 and February 1986, and diagnosed between January 1982 and June 1986.

Our approach to analysis has been to postulate simple mathematical forms (linear or exponential) for the growth in time of the number of infected individuals who will ultimately develop AIDS, and for the distribution (Weibull^{4,9} or gamma) of incubation period of those individuals. In some models a time-dependent probability of diagnosis has been included of probit form. This gives 4–6 unknown parameters to be estimated. An explicit expression for the log-likelihood of the data has been obtained and maximized numerically. Sensitivity to assumptions has been examined by comparing the maximized log-likelihoods achieved via different models.

The analysis has been done on the whole set of 297 patients and separately on males and females and on the data split by age at infection. The data were also artificially truncated at June 1985 and December 1985 to check for alterations in incidence of infective transfusions in 1985–1986.

The data are such that there is relatively direct information on the distribution of incubation time up to ~4–5 years, but beyond that one is extrapolating on the basis of the assumed mathematical form of the distribution. This implies that the mean, which depends critically on the upper tail of the distribution, is not a good indication of the information in the data. We wish therefore to emphasize the lower percentage points of the cumulative distributions (Fig. 1).

Our main conclusions are as follows. Exponential growth in infective transfusions fits better than linear growth. The doubling time of the exponential model is ~1 year, which is in agreement with the observed doubling time of seropositivity and incidence of AIDS in the whole population in a number of countries¹. The Weibull and gamma distributions provide equally good fits (Fig. 1 and Tables 1 and 2). The former distribution, which is widely used in survival studies, is equivalent to supposing that some power of incubation time is exponentially distributed: in the present case the power is roughly 2.5. Extrapolation of the gamma distribution generally indicates higher incidence of infection and longer incubation periods than does the Weibull. Without independent information, it is impossible to distinguish between the distributions in the current analysis. Division of

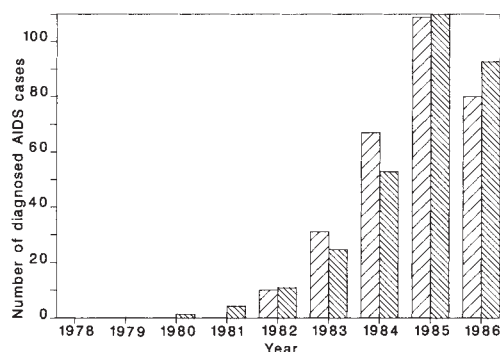


Fig. 2 A comparison of the total observed (left-hand bar of each pair) and expected (right-hand bar of each pair) numbers of AIDS cases diagnosed by year (regardless of year of transfusion). The expected numbers are derived from the model with exponential increase in the incidence of infective transfusions and a Weibull incubation period. Parameter values used (see legend to Fig. 1): $a = 14.48$, $b = 0.7072$, $p = 2.413$ and $q = 0.1384$. Note that the years are as defined in Table 1.

the data by sex showed the incubation time for females to be longer than for males. Notional mean (and median) for the females and males are 8.77 years (8.36 years) and 5.62 years (5.50 years) respectively (assuming an exponential rise in incidence and a Weibull distribution for the incubation period). The mean incubation time for children (0–4 yr at transfusion) is much shorter than for older patients (Fig. 1 and Table 2), which matches current perceptions of immunocompetence¹⁰. This does not preclude the possibility of paediatric cases having a long incubation, but shows that many cases have a much shorter incubation. The incubation period for patients older than 59 is less than that for the 5–59 years age group with means (and medians) of 5.50 (5.44) and 8.23 (7.97) respectively. This may arise as a consequence of high mortality unrelated to HIV infection in the years following transfusion amongst elderly patients. The doubling time of infective transfusions to children is higher than that to adults (1.3 years compared to 1.08 years). Artificial truncation of the data does not alter the form of the results. It does, however, decrease the doubling time of the childhood infections to that of the adults (0.94 years), which suggests some change in the infection process in 1985–86. This is explained by the introduction of screening for cytomegalovirus during the early 1980s in blood intended for infants, a process which coincidentally screens for HIV (T.A. Peterman, personal communication). The introduction of a diagnosis function is

just statistically significant, with the probability of correct diagnosis improving dramatically in 1982. We are concerned at overelaboration of analysis of limited data, however, and have not yet studied this further. Although, as emphasized above, there is no direct information about the form of the distribution much past 5 years, it is clear that under any reasonable assumption there is a long tail of values beyond 5 years, and that a substantial number of new cases of AIDS is likely to be observed in the future from those already infected in the particular population under study (Fig. 1).

In assessing these conclusions two important points should be noted. First, the distribution of incubation times in patients infected by blood transfusions is not necessarily the same as that in patients infected via other routes. Larger inocula of virus may be received via transfusion as compared with, for example, transmission via sexual activity. Second, as more data become available, it is important to update estimates of the distribution of incubation period, and in particular to study the relation with factors such as age, sex, ethnic/genetic background and route of transmission. It is also unknown how these results from an industrialized nation pertain to the developing world, where individuals are typically exposed more frequently and to a larger range of infectious agents. With the advent of mandatory screening of blood donations for seropositivity in USA and Europe, the USA transfusion-related AIDS cases are likely to remain a prime source of information on the incubation period for some time.

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Effect of immunization with a vaccinia-HIV *env* recombinant on HIV infection of chimpanzees

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Table 2 Expected and observed numbers of child patients by year of transfusion and diagnosis

Year of diagnosis	Year of transfusion							
	1978	1979	1980	1981	1982	1983	1984	1985
1978	0.00 (expected) 0 (observed)							
1979	0.07 0	0.03 0						
1980	0.10 0	0.21 0	0.06 0					
1981	0.07 0	0.29 0	0.44 0	0.13 0				
1982	0.02 0	0.17 1	0.62 0	0.92 2	0.27 1			
1983	0.00 0	0.05 0	0.36 1	1.29 1	1.94 2	0.57 2		
1984	0.00 0	0.01 0	0.10 0	0.76 0	2.70 2	4.06 4	1.19 0	
1985	0.00 0	0.00 0	0.01 0	0.15 0	0.98 1	2.94 5	3.22 2	0.24 0

As for Table 1, but only cases less than 5 years old at infection and who were diagnosed after June 1985 are included. Thus there are no figures for 1986, and the year 1985 runs from January to June only.

Acquired immunodeficiency syndrome (AIDS) is caused by human immunodeficiency virus (HIV) and is now recognized as a worldwide epidemic for which there is no cure or vaccine. Chimpanzees are the only other animals that can be infected by HIV^{1–4}, and therefore the chimpanzee-HIV model system is useful for testing potential HIV vaccines. However, with one exception¹, there have been no reports of clinical manifestations of AIDS in chimpanzees. We

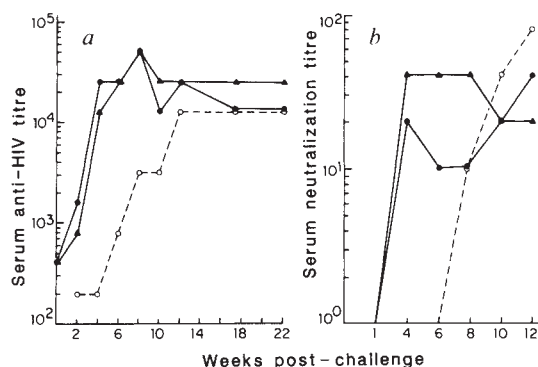


Fig. 1 *a*, HIV-specific antibody responses of v-HSVgD1-immunized (control, C-544, ○—○) and v-env5-immunized (C-552, ●—●; C-542, ▲—▲) chimpanzees following challenge with LAV-1. Serum samples obtained at different times after injection of LAV-1 were serially diluted and tested for HIV-specific antibodies using an HTLV-III EIA Kit (Abbott). Titres are expressed as the reciprocal of the highest dilution of serum that gave an absorbance above the cut-off value. *b*, HIV-neutralizing antibody titres in serum from chimpanzees following challenge. Titres are expressed as the reciprocal of the serum dilution which reduced the absorbance value by 50% compared to the value obtained in the absence of serum (see Table 1). Negative values are represented as unity.

Methods. Serum samples were heat-inactivated at 56 °C for 30 min, diluted in RPMI 1640 medium containing 10% fetal bovine serum, and mixed with an equal volume of virus containing 10 TCID₅₀ LAV-1. The mixtures were incubated for 45 min at 37 °C, then used to inoculate duplicate cultures containing 2 × 10⁵ CEM cells, which were held at 37 °C in humidified 5% CO₂ atmosphere for 12 days. The cells were collected, pelleted and lysed with 1% Triton X-100 in PBS, and viral antigen was assayed using a capture enzyme immunoassay (EIA). The EIA used monoclonal antibodies to p25 as capture reagents and horseradish peroxidase-conjugated human IgG purified from an HIV-seropositive person as signal antibodies.

report here results of an HIV vaccine trial in which nine chimpanzees were first immunized with either a recombinant vaccinia virus expressing the envelope glycoproteins of HIV strain LAV-1 (v-env5) or a control recombinant vaccinia virus and were then challenged with a high or low dose of LAV-1. Although HIV-specific antibody and T-cell responses were elicited by immunization, virus was isolated from lymphocytes of all challenged chimpanzees, indicating that immunization did not prevent infection by HIV. Among the animals that received a higher dose of LAV-1, one of two control chimpanzees, but none of the four v-env5-immunized chimpanzees developed substantial and persistent lymphadenopathy.

The chimpanzees were studied in two groups, one at Yerkes Primate Research Centre (two immunized and one control animal), the other at Southwest Foundation (four immunized and two controls). All chimpanzees were negative for antibodies to vaccinia virus and HIV, as determined by haemagglutination inhibition (HI) and enzyme immunoassay (EIA), respectively. This report details data obtained on the first group at Y.P.R.C. Chimpanzees C-542 and C-552 were vaccinated at one site on the upper back with 2 × 10⁸ plaque-forming units (PFU) of a recombinant of vaccinia virus (WR strain) that contains the entire LAV-1 *env* gene coding sequence (v-env5)^{5,6}. The control, C-544, received 2 × 10⁸ PFU of a recombinant vaccinia virus expressing the herpes simplex virus glycoprotein D gene (v-HSVgD1). The chimpanzees were boosted with 2 × 10⁸ PFU of v-env5 or v-HSVgD1 eight weeks later. Anti-vaccinia titres peaked (range, 80–320) ~2 weeks after the booster vaccination. Low concentrations of antibodies to HIV were detected by EIA in serum from chimpanzees C-542 and C-552 at 2 and 3 weeks after the boost, respectively. Immunoblots showed that these antibodies recognized the gp41 protein (data not shown). By 8 weeks after the boost, antibodies to gp120 were also detected,

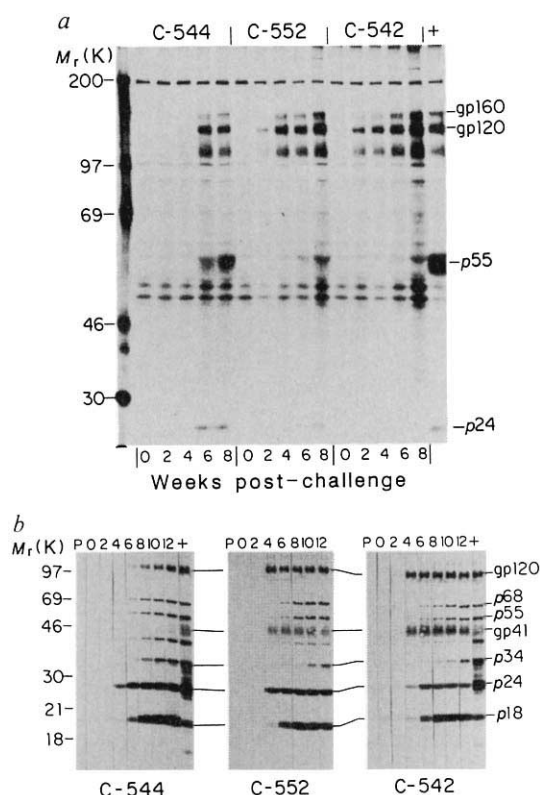


Fig. 2 *a*, Radioimmunoassay (RIP) of antibody responses to specific LAV-1 proteins in serum from immunized and control chimpanzees following challenge with LAV-1. Serum samples obtained at different times after virus challenge were assayed by RIP using LAV-1 persistently-infected HT cells that were radiolabelled with ³⁵S-methionine and ³⁵S-cysteine for 4 h. After lysing cells, cellular debris was removed by ultracentrifugation. Viral proteins were immunoprecipitated by overnight incubation at 4 °C with 10 µl of test serum, followed by incubation with Protein A-sepharose (Sigma) and centrifugation. Samples were separated on a 7.5% SDS-polyacrylamide gel and visualized by autoradiography. Molecular weight markers are shown on the left, and specific HIV *env* and *gag* gene products on the right. The band under the gp120 is a degradation product of the gp160/120. HIV-antibody-positive human serum is designated as +. *b*, Immunoblot analysis of HIV-specific antibody responses in serum from chimpanzees following LAV-1 challenge. Serum samples were diluted 100-fold in PBS containing 3% non-fat dry milk, and reacted with purified LAV-1 virion proteins previously resolved by SDS-PAGE on a 7-15% gradient gel and immobilized on nitrocellulose filters. HIV-specific antibodies that bound to LAV-1 proteins were detected by goat anti-human IgG antibodies conjugated to horseradish peroxidase. Serum samples tested included preimmunization (P), prechallenge (0), and biweekly blood samples following challenge (2–12). HIV-antibody-positive human serum from AIDS patients was used as positive control. Under reaction conditions optimal for detection of gp41 (ref. 6) antibodies to gp41 were detected in serum from C-542 and C-552 at prechallenge (not shown).

but not to the extent of antibodies to gp41. EIA antibody titres to HIV were 400 in all three chimpanzees, but the reactivity of C-544 was found by immunoblot to be nonspecific.

Despite the lack of detectable HIV neutralizing antibodies, all nine chimpanzees were challenged intravenously with 3.2 × 10⁵ or 100 50% tissue culture infectious dose (TCID₅₀) of LAV-1 for the following reasons. First, Moss *et al.*⁷ found that a single immunization of chimpanzees with a recombinant vaccinia virus expressing the hepatitis B surface antigen protected animals from infection despite the fact that very little or no antibody was detected. Second, we demonstrated HIV-specific helper and cytotoxic T cells in the v-env5-immunized chimpanzees⁸. Within

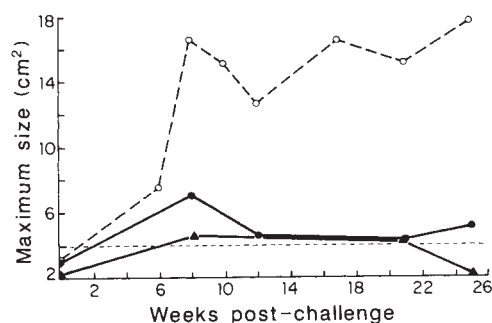


Fig. 3 Lymphadenopathy in vaccinated chimpanzees following challenge with 3.16×10^5 TCID₅₀ of chimpanzee-passaged LAV-1. Inguinal lymph nodes were measured with a metre rule; the areas in cm² of the largest nodes are plotted for C-544 (○---○), C-542 (▲—▲), and C-552 (●—●). C-544 and C-552 had slightly enlarged lymph nodes on the day of LAV-1 challenge; although the enlargements were not measured since they were of a size often observed in chimpanzees, the increased size is indicated by the placement of the lines at week 0. The horizontal line reflects the maximum amount of transient lymph node enlargement that, in our experience, is commonly seen in chimpanzees that are either HIV-infected or uninfected. For C-544, measurements of the right inguinal node are shown (average size, 3 cm × 5.5 cm), but the left node was only slightly smaller (average size, 2.5 cm × 5 cm).

Table 1 Development of neutralizing antibody to LAV-1 in challenged chimpanzees

Chimpanzee	Reciprocal of serum dilution	Absorbance* after weeks post-challenge with LAV-1						
		0	2	4	6	8	10	12
C-544 (Control)	10	0.777*	0.834	0.715	0.736	0.022	0.060	0.031
	20	0.744	0.746	0.723	0.673	0.703	0.041	0.044
	40	NT	0.776	0.466	0.609	0.614	0.363	0.073
	80	NT	0.890	0.649	0.765	0.799	0.703	0.184
	160	NT	0.845	0.801	0.765	0.704	0.787	0.728
	320	NT	0.845	0.808	0.720	0.801	0.805	0.788
C-552	10	0.812	0.762	0.024	0.036	0.213	0.021	0.018
	20	0.798	0.782	0.067	0.906	0.625	0.035	0.340
	40	NT	0.708	0.704	0.447	0.754	0.751	0.088
	80	NT	0.790	0.517	0.676	0.798	0.728	0.525
	160	NT	0.783	0.727	0.876	0.813	0.730	0.746
	320	NT	0.783	0.673	0.976	0.748	0.791	0.734
C-542	10	0.780	0.721	0.227	0.026	0.03	0.020	0.027
	20	0.782	0.673	0.375	0.029	0.482	0.019	0.173
	40	NT	0.700	0.096	0.394	0.327	0.499	0.439
	80	NT	0.846	0.759	0.643	0.719	0.507	0.630
	160	NT	0.784	0.653	0.872	0.754	0.804	0.783
	320	NT	0.740	0.789	0.724	0.855	0.804	0.839

* Absorbance values represent the average of four data points.

Underlined numbers represent $\geq 50\%$ reduction of absorbance compared to infected-culture controls. Absorbance of infected cultures was 0.780 and 0.030 for non-infected cultures.

NT, not tested. Experiments were performed as described in legend to Fig. 1b.

2 weeks of challenging all three Yerkes chimpanzees with 3.2×10^5 TCID₅₀ of LAV-1, LAV-1 antibody titres in both v-env5 immunized chimpanzees increased, and were maximal at 6 weeks post-challenge (Fig. 1a). In contrast, LAV-1-specific antibody in the control animal, C-544, was undetectable until 6 weeks and did not reach a maximum until 12 weeks after challenge. Similarly, HIV-neutralizing antibodies were detected in both v-env5-immunized animals by 4 weeks after challenge but not in C-544 until 8 weeks (Table 1 and Fig. 1b). By 2 weeks after challenge, both v-env5-immunized animals had large amounts of antibody to gp160/120 (Fig. 2a) and gp41 (Fig. 2b) which was not attained by C-544 until 6 (Fig. 2a) and 12 (Fig. 2b) weeks, respectively. In all three animals, antibodies to gag antigens (p55, p24, p18) were detected 4–6 weeks after challenge (Fig. 2b). These results indicate that animals immunized with v-env5 were specifically primed for developing anti-

bodies to HIV env antigens, which correlated with neutralizing antibodies.

Following challenge with LAV-1, chimpanzee peripheral blood mononuclear cells (PBMC) were co-cultured with PHA-stimulated human PBMC. HIV was isolated from PBMC of all animals by 2 or 3 weeks after challenge; maximal numbers of infected cells were reached at 4–8 weeks and ranged from 10^3 – 10^4 per 10^7 total PBMC. There was thus no apparent difference in the ability of LAV-1 to establish infection in chimpanzees previously immunized with either v-env5 or v-HSVgD1.

Following challenge with LAV-1, there were no signs or symptoms of disease in eight of nine chimpanzees. But within 6 weeks of LAV-1 challenge, one (C-544) of two control chimpanzees given 3.2×10^5 TCID₅₀ developed lymphadenopathy in both inguinal and axillary lymph nodes. Inguinal nodes increased to approximately 2–3 cm × 5.5 cm by 8 weeks post-challenge, and they have remained at that approximate size for over 8 months (Fig. 3). Biopsy revealed the marked follicular hyperplasia and irregularly shaped germinal centres characteristic of lymphadenopathy in HIV-infected humans⁸; LAV-1 was isolated from the enlarged node. The recipients of v-env5 showed a milder degree of lymphadenopathy with inguinal nodes in chimpanzee C-552 measuring up to 2 cm × 3.5 cm which resolved by 4 months and was comparable to transient enlargements we have observed³ previously in LAV-1-infected chimpanzees.

This is the second report of prolonged and substantial lymphadenopathy in a chimpanzee following infection with HIV. The lymphadenopathy may have resulted from the amount of virus inoculated (3.2×10^5 TCID₅₀), the largest documented dose of HIV given to a chimpanzee. In the first report by Alter *et al.*¹, a chimpanzee received human plasma pooled from lymphadenopathy and AIDS patients. The virus titre administered was probably considerable because of the large volume (450 ml) of plasma given.

While immunization of chimpanzees with v-env5 did not prevent HIV infection, it may have affected the development of lymphadenopathy, since no v-env5-immunized animal developed lymphadenopathy to the same extent as one of the control chimpanzees. Along these lines, immunization of mice with a recombinant vaccinia–Friend leukaemia virus protected mice from developing splenomegaly, but did not prevent virus infection¹⁰. Considering the relevance of our results to vaccine trials in humans, it is not known whether immune responses generated in humans would be similar to those we have detected in chimpanzees. The level of immunity generated with other antigens differs among strains of mice and among species. It has been recently reported¹¹ that immunization of humans with a similar recombinant vaccinia–HIV env virus resulted in both HIV neutralizing antibodies and HIV-specific T cell-mediated immunity.

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Major histocompatibility complex class I molecules internalized via coated pits in T lymphocytes

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Endocytosis of the major histocompatibility complex (MHC)-encoded class I and class II molecules has been the subject of recent investigations. Class I molecules, which are key elements in T cell-mediated cytotoxicity¹, are differentially endocytosed by different cell types. Fibroblasts internalize their class I molecules via uncoated cell surface vesicles and tubular invaginations when these molecules are cross-linked with multivalent ligands^{2,3}. T lymphocytes internalize their class I molecules spontaneously, but B lymphocytes do not internalize them at all⁴⁻⁶. Here we describe a morphological investigation of the mechanism by which class I molecules are endocytosed by T lymphocytes. We show that, unlike fibroblasts, T lymphocytes spontaneously internalize 20–40% of their class I molecules in a process involving coated pits and coated vesicles. Thus, the endocytic pathway of class I molecules in T lymphocytes is similar to those of other more classical cell-surface receptors involved in receptor-mediated endocytosis⁷. In contrast, the same class I molecules remained on the cell surface in B lymphocytes. These data show that class I molecules are differentially regulated in T and B lymphocytes and fibroblasts.

T and B spleen lymphocytes from CBA/J mice (H-2^k haplotype) were enriched and analysed by cytofluorimetry after staining with monoclonal antibodies. The results are summarized in Table 1. After enrichment for T cells, at least 90% of the cells specifically bound the anti-Thy 1 antibody, and in the preparation enriched for B cells, 93% of the cells specifically bound the anti-H-2IE antibody. Both cell types expressed H-2K molecules. The same phenotypic distribution was observed after stimulation of the cells by mitogen for blast induction.

Visualization of the endocytic pathway of the H-2K molecules by electron microscopy was achieved using a monoclonal anti-H-2K^k antibody (H100-5/28)⁸, in conjunction with protein A gold particles of mean diameter 15 nm as the electron-opaque markers. T cells maintained at 4 °C had a heterogeneous distribution of gold beads on their surface. Beads were found singly and in clusters around the cell perimeter (Fig. 1a) and no beads were seen in the interior of the cells (Table 2). Binding of protein A gold particles was not observed when the cells were incubated with an irrelevant antibody (B1.23.2, anti-HLA)⁹ as control (not shown). Coated pits of mean diameter 150 nm were sparsely distributed, occurring with a frequency of once in every 3 nm² of cell surface. Coated vesicles were also relatively infrequent in stimulated T cells, appearing with a frequency of ~7–9 per nm³. About half of the coated pits that were seen in T-cell preparations had one or more beads associated with them (Fig. 1b).

After 30 seconds of warming, a few beads were seen inside T cells but the configuration on the outside of the cells was identical to that seen on cells maintained at 4 °C. After 1 min, more beads were seen in the cell interior, 40% of them in coated vesicles (Fig. 1c): 28% of the coated vesicles contained beads after one minute at 37 °C (Table 2). With longer incubation (1–5 minutes) the beads accumulated in large and smooth endocytic

Table 1 Fluorescence analysis of T and B spleen lymphocyte populations after enrichment

Monoclonal antibodies	Percentage of positive cells			
	T cells	B cells	T blasts	B blasts
Anti-H-2IE ^k	2.0	93.0	5.8	96.2
Anti-Thy 1.2	90.0	1.0	95.4	10.3
Anti-H-2K ^k	96.0	98.0	98.5	99.0
Anti-HLA	1.8	9.0	3.7	12.0

Anti-H-2IE^k (H39.352.4)¹⁶, anti-H-2K^k (H100-5/28)⁸ and anti-HLA B, C (B1.23.2)⁹ are mouse monoclonal IgG2a, κ antibodies. Anti-Thy 1.2 (30-H12)¹⁷ is a rat monoclonal IgG2b antibody. Spleen cells from CBA/J mice were treated with 0.83% NH₄Cl to remove erythrocytes. T lymphocytes were obtained by nylon wool filtration¹⁸ and depleted of residual B cells by treatment with the anti-H-2IE^k antibody and complement. B cells were recovered from the nylon wool columns and depleted of residual T cells by treatment with the anti-Thy 1 antibody and complement. Viable cells were recovered by centrifugation over a Ficoll gradient. For blast induction, cells (30 × 10⁶) were incubated at 37 °C in 7% CO₂ in tissue culture flasks with 20 ml of RPMI 1640 medium supplemented with 5% heat-inactivated fetal calf serum, 2 mM glutamine, 5 × 10⁻⁵ M 2-mercaptoethanol and gentamicin. T lymphocytes were incubated for 40 h with 2 µg ml⁻¹ Concanavalin A (Con A; Miles). B lymphocytes were incubated for 40 h with 20 µg ml⁻¹ of lipopolysaccharide from *Escherichia coli* (Difco). Flow cytometry data were obtained by using an EPICS V cytofluorimeter, after staining of the cells with FITC-labelled antibodies.

vesicles defined as endosomes^{10,11} (Fig. 1d). Acidification of the endocytic vesicles occurs during this process^{5,12,13}. Prolonged incubation (up to 10 hours) led to the appearance of increased number of beads in the cell interior, most of them in lysosomes (Fig. 1e) which also contained electron dense debris, with very few in coated vesicles (Table 2). Those beads that remained on the cell surface after 10 hours were mainly in large clusters as if capping had occurred; very few beads were found individually or in small clusters. The observation that a significant fraction of the beads remain on the cell surface is consistent with kinetic experiments showing that only a fraction (20–40%) of surface bound anti-H-2K antibodies are internalized by T cells (see Fig. 2), supporting the view that H-2K molecules are heterogeneous with respect to endocytosis.

No protein A gold particles were seen in smooth cell-surface invaginations, suggesting that endocytosis of H-2K molecules in T lymphocytes occurs only via coated pits. The small diameter of coated pits (estimated at 150 nm) and coated vesicles limits the size of the particle that lymphocytes can endocytose via this pathway. This is in agreement with previous results from our

Table 2 Cellular distribution of H-2K molecules on T and B lymphocytes using anti-H-2K antibody and 15-nm protein A gold beads

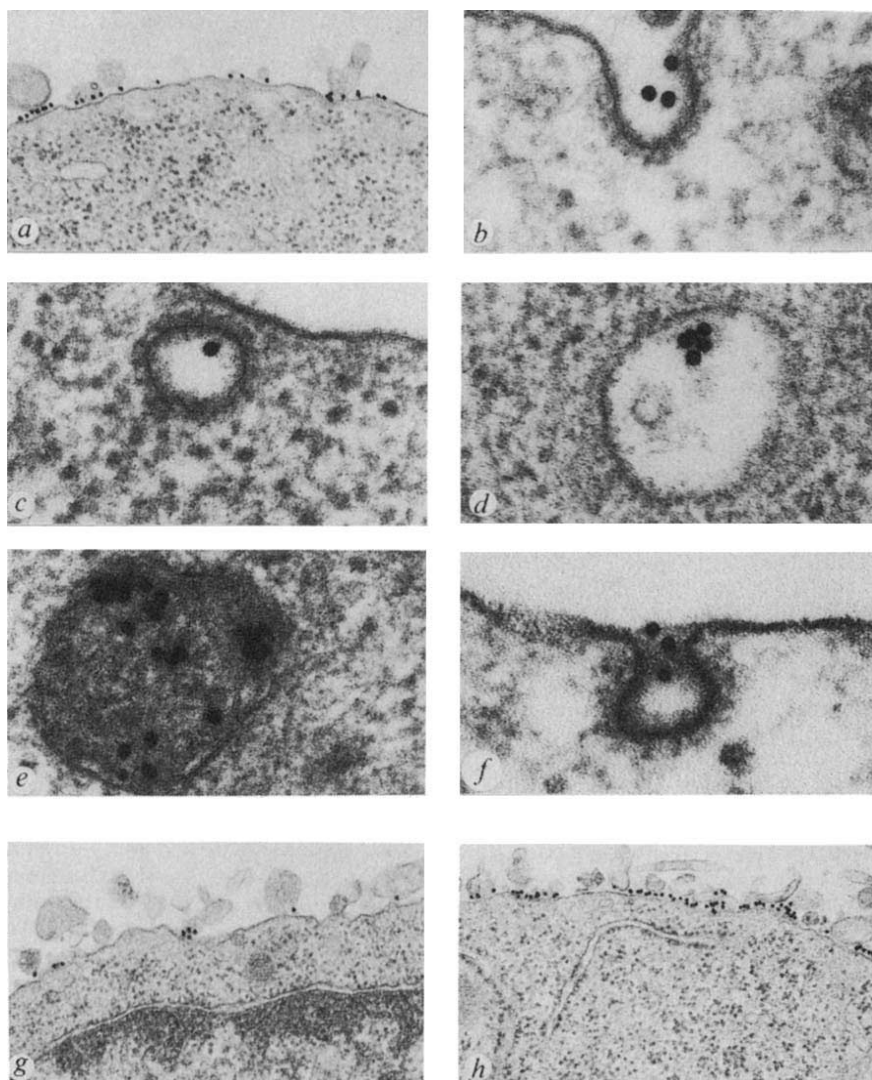
	Time	Beads counted per specimen	Beads on surface per µm ²	Beads inside cells per µm ³	% Coated vesicles occupied
T cells					
	0	325	48.3	0.3	0
	30 s	321	48.8	1.5	0
	1 min	318	51.1	9.6	28.0
	10 h	1,351	57.3	76.0	2.6
B cells					
	0	321	88.4	14.4*	0
	30 s	561	46.6	12.1*	0
	1 min	544	91.1	16.5*	0
	10 h	785	66.8	20.3*	0

Stereological analysis: consecutive cells were photographed at ×14,000 and printed at ×35,000 to permit enumeration of beads. Stereological measurements were made according to Weible¹⁹.

* Beads in deep plasma membrane invaginations. See text for details.

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Fig. 1 Electron micrographs of the endocytic pathway of H-2K molecules. Lymphoblasts (5×10^5) were incubated with $40 \mu\text{g ml}^{-1}$ of anti-H-2K^k antibody for 1 h at 4 °C. After two washes with cold culture medium, cells were incubated for 2 h at 4 °C with 50 μl of protein A gold (mean diameter 15 nm; Janssen Life Sciences Products). Cells were washed twice with the same medium and incubated either at 4 °C or 37 °C. At different intervals of time, the cells were fixed for 2 h in 0.1 M cacodylate buffer, pH 7.4, containing 5% sucrose, 2.0 mM CaCl_2 and 2.0% glutaraldehyde and then washed three times in the same buffer without glutaraldehyde. *a*, Surface-bound protein A gold particles when T lymphoblasts were maintained at 4 °C. *b*, Protein A gold particles attached to H-2K molecules via the anti-H-2K antibody located in coated pits. *c*, After 1 min of incubation at 37 °C, particles were inside coated vesicles. *d*, After 2 min, particles were concentrated into large uncoated endocytic vesicles. *e*, After 15 min, particles were found in lysosome-like structures containing cell debris. *f*, T lymphoblasts were fixed with 0.5% paraformaldehyde before staining in ice. Some protein A gold particles can already be seen within coated pits. *g*, Surface-bound protein A gold particles when B lymphoblasts were maintained at 4 °C. *h*, After 1 h of incubation at 37 °C capping of H-2K molecules was observed. No beads were detected in the cytoplasm for the majority of the cells. Magnification: *a*, *g*, $\times 33,600$, *b-f*, $\times 151,200$.



laboratory showing that T lymphocytes are incapable of internalizing H-2K molecules when particles larger than 100 nm diameter are bound with specific antibody¹⁴.

Thus, in T cells, H-2K antigens are not exclusively present on coated pits but none the less, the cells appear to internalize these molecules via coated pits and vesicles. Perhaps coated pits form subjacent to bound ligand and rapidly pinch off to form coated vesicles. This hypothesis is based on the observation that more than one quarter of the coated vesicles are labelled with beads within 20 seconds without the appearance of a corresponding population of labelled pits, suggesting that coated pits have a very ephemeral existence and that vesicles have a longer lifespan than the pits. Ligands bound to H-2K molecules are not recycled back to the surface after internalization and, as shown in Fig. 1*e*, end up in lysosomes.

The same pattern of endocytosis has been observed with other strains of mice such as AKR and B10.A. These data were obtained with Con A stimulated T lymphocytes. Con A is known to bind to glycoproteins, including those of the MHC¹⁵. To rule out the possibility that what we observed was due to Con A binding to class I molecules, we performed similar experiments on the RDM4 thymoma cell line which is of AKR origin. The same pathway of endocytosis was observed and only a small proportion of the H-2K molecules were internalized.

H-2K molecules could also be localized to coated pits in T cells pre-fixed with paraformaldehyde (Fig. 1*f*). Similar results were also obtained with the RDM4 thymoma line and Con A-stimulated T lymphocytes. Such a location for the antigen is

compatible with constitutive internalization of a proportion of these molecules.

B cells had even fewer coated pits and vesicles than T cells. The frequency of pits was 1 per 4.5 nm^2 of surface boundary and vesicles had a frequency of 1–2 per nm^3 . As with T cells, beads were initially distributed individually and in clusters over the cell surface (Fig. 1*g*). However, unlike the situation with T cells, gold beads were never seen in association with either coated pits or vesicles in these cells (Table 2). An unexpected finding was a high frequency of apparently internalized beads in some cells at time zero at 4 °C. These beads were not all near the surface where they could be interpreted as due to grazing sections of shallow membrane invaginations. But the fact that they did not accumulate over time inside the cells and particularly in lysosomes suggests that the beads are entering a deeply invaginated compartment that is continuous with the plasma membrane. At the end of 10 hours, the beads on the outside of the cells were in large aggregates as if capping had occurred (Fig. 1*f*). Most of the cells had no beads inside; accumulation of beads into lysosomes occurred in only 15% of the cells examined suggesting that these cells represent contaminating T cells (see Table 1).

To quantify the amount of the anti-H-2K antibody internalized with H-2K molecules by T cells, cells were incubated with the antibody, washed free of unbound material and then incubated for different times at 37 °C or 4 °C. The amount of antibody remaining on the surface was determined by incubation with radiolabelled protein A. Figure 2 shows that the cells internalized

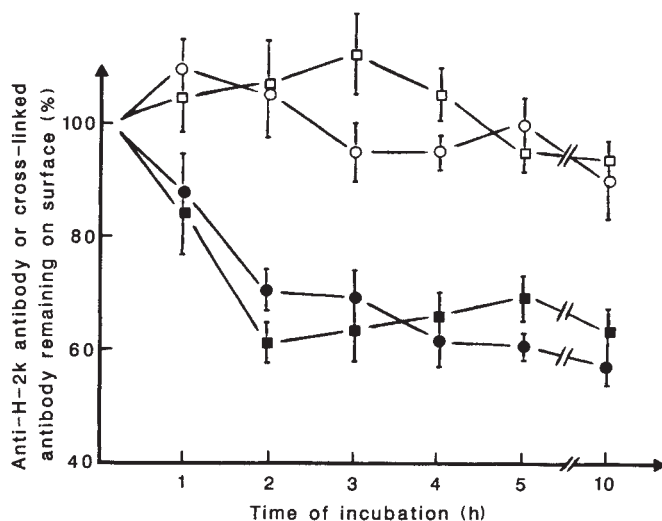


Fig. 2 Disappearance of surface-bound anti-H-2K antibody from T lymphoblasts. Aliquots of cells (5×10^5) were incubated at 4°C for 1 h with the anti-H-2K antibody ($40 \mu\text{g ml}^{-1}$), then washed twice with cold medium and incubated at 4°C (○) or 37°C (●). After different intervals of time, cells were incubated with 2×10^5 c.p.m. of ^{125}I -labelled protein A (NEN) for one additional hour at 4°C. Cells were washed twice with cold medium and the cell-associated radioactivity in the pellet counted. Radioactivity bound to the cells pre-incubated with the anti-H-2K antibody at time 0 was $26,000 \pm 3,200$ c.p.m., and that bound to the cells pre-incubated with the anti-HLA antibody as control at time 0 was 500 ± 200 . For each plotted point, background obtained with the control anti-HLA antibody has been subtracted. In a different experiment the cells were incubated with the anti-H-2K antibody as described above, then incubated for an additional hour at 4°C with $25 \mu\text{l}$ of a rabbit antiserum to mouse IgG2a (Bionetics) as cross linker. After washing, the cells were incubated at 4°C (□) or 37°C (■). After different intervals of time, cells were treated with ^{125}I -labelled protein A as described above. Radioactivity bound to the cells pre-incubated with the anti-H-2K antibody at time 0 was $52,000 \pm 3,700$ c.p.m., and that bound to the cells pre-incubated with the anti-HLA antibody as control at time 0 was $1,500 \pm 700$ c.p.m. For each plotted point, background obtained with the control anti-HLA antibody has been subtracted. Bars represent the mean value $\pm$ s.d. of three separate experiments.

the antibody only at 37°C, suggesting an active endocytic pathway. The disappearance of antibody levelled off after 2 hours. Cross-linking of H-2K molecules by anti-H-2K and a rabbit anti-mouse IgG2a had no effect (Fig. 2). No more than 20–40% of the antibody was internalized with a half life of 1 hour, confirming the results of the ultrastructural studies.

So far, only receptors for specific ligands have been shown to enter the cells via this endocytic pathway⁷. This observation can now be extended to MHC class I molecules. Although no receptor function with a specificity for a ligand has yet been claimed for these molecules, the reasons that T lymphocytes spontaneously internalize only a fraction of their class I molecules via coated pits, whereas B lymphocytes do not even after cross-linking and fibroblasts internalize all of their class I molecules via uncoated cell surface invaginations and only when these molecules are cross-linked, remains intriguing.

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Activation of a transposable element in the germ line but not the soma of *Caenorhabditis elegans*

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The genetic activity of transposable elements is tightly controlled in many species. Transposons that are relatively quiescent under certain circumstances can excise or transpose at greatly increased rates under other circumstances. For example, 'genomic shock' can activate quiescent maize transposons^{1–3}, 'cytotype' and tissue-specific splicing regulate *Drosophila* P factors^{4–6}, copy number controls Tn5 transposition in bacteria^{7,8}, and developmental timing affects the production of transposon-like intracisternal A-particles in mouse embryos^{9–11}. The *Caenorhabditis elegans* transposable element Tc1 is subject to both strain-specific and tissue-specific control. Multiple copies of Tc1 are present in the genome of all *C. elegans* strains collected from nature. However, these elements are genetically active in only certain isolates. For example, in *C. elegans* variety Bristol transposition and excision of Tc1 are undetectable, but in variety Bergerac transposition and excision are frequent^{12,13,14}. Moreover, in variety Bergerac, Tc1 is about 1,000-fold more active in somatic cells than in germ cells^{13,15}. We have investigated the genetic basis for the germ/soma regulation of Tc1 activity. We have isolated mutants that exhibit increased frequencies of Tc1 excision in the germ line. The frequencies of Tc1 excision in the soma are unaltered in these mutants. These mutants also exhibit high frequencies of Tc1 germ-line transposition, and this results in a mutator phenotype. Nearly all mutator-induced mutations are caused by insertion of Tc1.

Strain TR445 (genotype *unc-54(r323::Tc1)*, from the Bergerac genetic background) contains a Tc1 insertion in the *unc-54* myosin heavy chain gene. Because of this, it is muscle defective and uncoordinated. Tc1 is genetically active in strain TR445, and motile, wild-type revertants occur spontaneously at a frequency of 1×10^{-5} . Such revertants result from excision of Tc1 (ref. 13). We identified mutant derivatives of TR445 that have increased frequencies of reversion. TR445 young adults were mutagenized with ethylmethane sulphonate (EMS), and one F₁ offspring was placed on each of ~1,500 Petri dishes. We screened these populations three to four generations later, and retained those that contained an unexpectedly large number of wild-type revertants. Wild-type revertants were picked, and among their self-fertilized offspring *unc-54⁻::Tc1* homozygotes were identified. We retested the mutants, and those consistently showing elevated reversion frequencies were isolated singly until a homozygous frequently reverting stock was obtained. We term these stocks *mutator* mutants (see below), and the mutations causing this phenotype define *mut* genes. Table 1, column A,

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Table 1 Frequency of germ-line and somatic reversion of the Tc1-induced mutation *unc-54(r323)* in mutator mutants

Strain	Genotype	A	B
		Frequency of <i>unc-54⁺</i> germ-line revertants	Frequency of <i>unc-54⁺</i> somatic revertants
TR445	<i>unc-54 (r323::Tc1)</i>	1×10^{-5}	1.7%
TR674	<i>unc-54 (r323::Tc1) mut-2(r459)</i>	1×10^{-3}	1.1%
TR673	<i>unc-54 (r323::Tc1) mut(r458)</i>	1×10^{-3}	0.9%
TR675	<i>unc-54 (r323::Tc1) mut(r460)</i>	1×10^{-3}	2.3%
TR630	<i>unc-54 (r323::Tc1) mut-3(r456)</i>	9×10^{-5}	0.9%
TR628	<i>unc-54 (r323::Tc1) mut(r454)</i>	1×10^{-4}	1.2%
TR629	<i>unc-54 (r323::Tc1) mut(r455)</i>	1×10^{-4}	1.3%
TR631	<i>unc-54 (r323::Tc1) mut(r457)</i>	1×10^{-4}	1.3%
TR689	<i>unc-54 (r323::Tc1) mut(r461)</i>	4×10^{-5}	ND

Germ-line revertant frequencies were determined by establishing a series of independent cultures for each strain and allowing them to grow to a measured population size. Populations were then screened for the presence or absence of wild-type revertants. The proportion of cultures not showing revertants (P_0) was used to calculate the revertant frequency using the Poisson distribution ($P_0 = e^{-aN}$; a , revertant frequency; N , population size). This method corrects for jackpots and multiple revertants occurring in a single culture. For most measurements, $N = 12,000$. P_0 values were as follows: TR445, 525/595; TR630, 5/15; TR628, 4/15; TR629, 4/15; TR631, 3/15; TR689, 22/36. For other strains the values were: TR673, 0/10 ($N = 3,500$) and 6/10 ($N = 350$); TR674, 0/10 ($N = 3,500$) and 6/10 ($N = 350$); TR675, 0/10 ($N = 3,500$) and 6/10 ($N = 350$). Somatic revertant frequencies were determined by counting the broods of 30–50 animals. Egg-laying mosaic revertants were scored among these broods; each of the frequencies is based upon 8–20 independent mosaic revertants. ND, not done.

shows that mutator mutants have germ-line reversion frequencies that are 4–100-fold higher than that of their parent strain TR445. We investigated the *unc-54* gene structures of 21 mutator-induced wild-type revertants using genomic Southern blots. In all cases, the revertants result from precise or nearly-precise excision of Tc1. We isolated eight mutator mutants among approximately 1,500 offspring of EMS-mutagenized TR445. We isolated no mutator mutants among 1,095 offspring of unmutagenized TR445. Thus, mutations causing a mutator phenotype are EMS-induced ($P > 0.98$ using Fisher's exact test).

The effects of mutator mutations on Tc1 excision are confined to the germ line. Excision of the *unc-54(r323::Tc1)* insertion in certain somatic lineages results in mosaic revertants that have a characteristic phenotype¹³. Such animals lay eggs because the revertant clone of cells includes the sex muscles. Table 1, column B, shows that the frequencies of egg-laying, mosaic revertants of mutator strains are equal to that of their parent strain TR445.

Mutator mutants show increased frequencies of Tc1 transposition in the germ line, even though their isolation was based solely on a phenotype of increased Tc1 excision in the germ line. We define Tc1 transposition as insertion of elements at new chromosomal positions. To measure transposition, we determined the frequency of Tc1-induced spontaneous mutations affecting the *unc-22* gene. Table 2, column A, shows that mutator mutants exhibit *unc-22* spontaneous mutant frequencies that are 4–40-fold higher than that for *C. elegans* variety Bergerac, the standard laboratory strain in which Tc1 is active. Mutator mutants having the highest frequencies of Tc1 excision (see Table 1) also have the highest frequencies of Tc1 transposition.

Most mutator-induced *unc-22* mutations are caused by Tc1 insertion. We performed total genomic Southern blots of 24 mutator-induced *unc-22* mutants. Figure 1 shows a representative experiment, and Table 2, column B, summarizes the results.

Of 24 mutants tested, 22 contain insertions in the *unc-22* gene; 20 of these insertions have the properties expected for Tc1. We infer that Tc1 causes a mutation if the *unc-22* gene contains an insertion of $1,600 \pm 50$ base pairs relative to wild-type and if the inserted DNA is cut with restriction enzyme *EcoRV* but not with enzymes *BglII* and *SacI*. The sequence of Tc1 predicts such restriction site data¹⁶. Although these criteria do not prove rigorously that an insert is Tc1, all similar mutations affecting the *unc-54* gene are caused by Tc1 (11 of 11; see ref. 13). Of the four mutants that do not contain Tc1 insertions, two have a wild-type Southern blot pattern and two contain insertions of 2.0–2.5 kb. The relationship of these insertions to Tc1 is unknown.

In addition to their phenotypes involving Tc1 excision and

Table 2 Frequency of spontaneous *unc-22⁻::Tc1* mutations and spontaneous males in mutator mutants

Strain	Genotype	A	B	C
		Frequency of spontaneous <i>unc-22</i> mutants	Fraction of <i>unc-22</i> mutations caused by Tc1	Frequency of male offspring (no. of males)
EM1002	WT variety Bergerac	5×10^{-5}	13/15	ND
TR644	WT revertant of TR445	$< 2 \times 10^{-5}$	—	0.15% (4)
TR679	<i>mut-2(r459)</i>	2×10^{-3}	5/5	1.7% (14)
TR676	<i>mut(r458)</i>	$> 6 \times 10^{-4}$	3/4	2.1% (10)
TR682	<i>mut(r460)</i>	$> 7 \times 10^{-4}$	3/4	3.0% (9)
TR638	<i>mut-3(r456)</i>	8×10^{-4}	3/3	1.5% (59)
TR632	<i>mut(r454)</i>	$> 3 \times 10^{-4}$	1/1	2.8% (52)
TR635	<i>mut(r455)</i>	$> 3 \times 10^{-4}$	2/2	3.3% (41)
TR641	<i>mut(r457)</i>	2×10^{-4}	2/3	1.6% (27)
TR690	<i>mut(r461)</i>	3×10^{-4}	1/2	0.8% (3)

WT, wild-type. The frequency of spontaneous *unc-22* mutants was determined by establishing a series of independent synchronized cultures for each strain and allowing them to grow to a measured population size. Populations were then screened for presence or absence of *unc-22* mutants based upon their phenotype of nicotine-induced twitching²⁰. This method detects both heterozygous and homozygous *unc-22* mutants. The proportion of cultures not showing mutants (P_0) was used to calculate the spontaneous mutant frequency using the Poisson distribution ($P_0 = e^{-aN}$; a , mutant frequency; N , population size). Strains TR644, TR679, TR676, TR682, TR638, TR632, TR635, TR641 and TR690 are spontaneous wild-type revertants of strains TR445, TR674, TR673, TR675, TR630, TR628, TR629, TR631 and TR689, respectively (see Table 1). Data for frequency calculation: for EM1002 $P_0 = 16/29$, $N = 13,200$; for TR644 $P_0 = 8/8$, $N = 6,200$; for TR679 $P_0 = 4/14$, $N = 520$; for TR676 $P_0 = 0/6$, $N = 1,630$; for TR682 $P_0 = 0/7$, $N = 1,470$; for TR638 $P_0 = 3/8$, $N = 1,210$; for TR632 $P_0 = 0/8$, $N = 3,380$; for TR635 $P_0 = 0/8$, $N = 2,880$; for TR641 $P_0 = 3/8$, $N = 4,380$; for TR690 $P_0 = 6/8$, $N = 900$. Strain EM1002 is included for comparison, because TR445 (and its wild-type revertant TR644) have slightly lower frequencies of Tc1 excision and transposition than standard Bergerac strains. TR445 and, hence, all mutator mutants described in this paper have a Bristol/Bergerac hybrid genetic background. *C. elegans* variety Bristol, the standard laboratory strain, is inactive for Tc1 germ-line transposition²¹ and excision¹². *C. elegans* variety Bergerac, however, is active for both^{12,13}. We derived strain TR445 by crossing the predominantly Bergerac strain TR397 (genotype *unc-105(n490) unc-54(r323::Tc1)*; see ref. 13) once with wild-type Bristol males. An *unc-54(r323::Tc1)* homozygote (TR445) was isolated among the self-fertilized offspring of the resulting heterozygote. We grew TR445 for many generations and repeatedly cloned it before using it in the mutator screen. Thus, TR445 contains a unique combination of Bristol and Bergerac genomes, but it is homozygous at all loci. The frequencies of Tc1 transposition and excision are slightly lower in TR445 than in true Bergerac. For example, wild-type revertants of *unc-54(r323::Tc1)* occur at a frequency of 3×10^{-5} in the Bergerac genetic background, but 1×10^{-5} in the TR445 genetic background. The fraction of *unc-22* mutations caused by Tc1 insertion was determined using total genomic Southern blots²² of wild-type and mutant DNAs (see Fig. 1). Probes were λ phages DM17 and DM18 (ref. 16), representing ~25 kb of the *unc-22* gene region. These probes detect all Tc1 insertions that cause a strong *unc-22* mutant phenotype (D. Moerman, personal communication). The frequency of male offspring was determined by counting the progeny of 10–80 hermaphrodite broods. The number of male offspring is shown in parentheses.

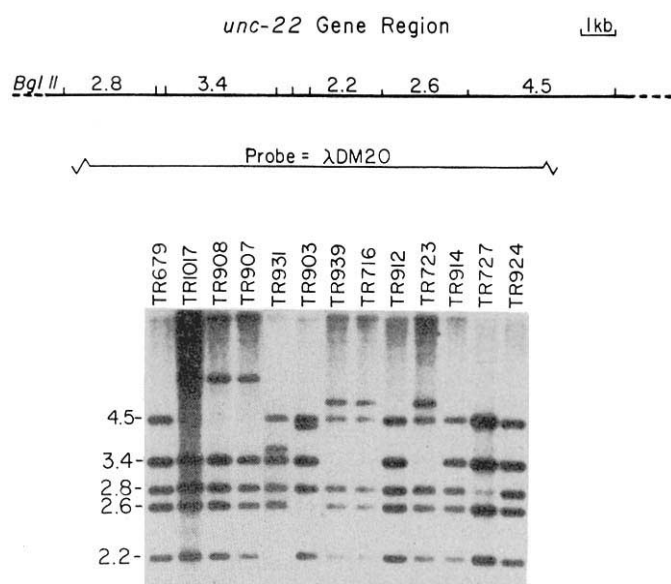


Fig. 1 Southern blot analysis of mutator-induced *unc-22* mutations and their revertants. Each *unc-22* mutation results from an insertion of ~1.6 kb, and each revertant no longer contains the inserted material. Total genomic DNAs were digested with *Bgl*II and the restriction fragments were separated on a 0.8% agarose gel. The radiolabelled probe DNA was recombinant λ phage DM20, which contains ~14 kb of genomic material from the *unc-22* gene region¹⁴. Strain TR679 (genotype *mut-2(r459)*) shows the wild-type pattern of *unc-22* restriction fragments. Strains TR1017, TR908, TR907, TR931, TR903, TR939, TR716, TR723 and TR727 each contain a spontaneous *unc-22* mutation induced by *mut-2(r459)*, *mut-2(r459)*, *mut-2(r459)*, *mut-2(r458)*, *mut-2(r457)*, *mut-3(r456)*, *mut-3(r456)* and *mut-3(r456)*, respectively. For each restriction fragment affected by *Tc1* insertion, the 'empty site' products of somatic excision are detected as a minor fraction of DNA having wild-type mobility¹⁵. Strains TR912, TR914 and TR924 are spontaneous *unc-22*⁺ revertants of strains TR716, TR723 and TR727, respectively.

transposition, mutator mutants show increased frequencies of X-chromosome nondisjunction. In *C. elegans*, sex is determined by a chromosomal XX/XO mechanism¹⁷. The proportion of male offspring arising from a hermaphrodite parent is a measure of the X-chromosome nondisjunction frequency¹⁸. Table 2, column 3, shows that each mutator mutant has a mild Him (high incidence of males) phenotype. We do not know if the Him phenotype is a direct result of high levels of *Tc1* transposition and excision, or whether it is due to pleiotropy of the *mut* mutations. We tested whether existing *him* mutants¹⁸ are active for *Tc1* transposition. If *him* mutants have been activated for *Tc1* transposition, then the number and/or position of *Tc1* elements in their genomes should be altered. We performed total genomic Southern blots of *him-1(e879)*, *him-2(e1065)*, *him-3(e1147)*, *him-4(e1267)*, *him-5(e1467)*, *him-6(e1423)*, *him-7(e1480)* and *him-8(e1489)* using a *Tc1* hybridization probe. The number and position of *Tc1* elements in these strains are identical to that of the wild-type Bristol strain from which they were derived.

The number of different loci that can be mutated to yield a mutator phenotype is unclear. The Him phenotype of mutator mutants is semi-dominant, and this makes interpretation of complementation tests difficult. Genetic mapping experiments demonstrate that *mut-3(r456)* is linked to *unc-54* on chromosome I but that *mut-2(r459)* is not (data not shown). At least two loci, therefore, are represented among our mutants. We recovered mutator mutations at a frequency that is equal to or greater than the frequency of EMS-induced mutations affecting an average sized *C. elegans* gene¹⁹. This high frequency suggests that mutator mutations are loss-of-function alleles. Yet the semi-

dominance of the Him phenotype suggests that mutator mutations are gain-of-function alleles. We suggest two explanations for these observations. Firstly, the genes affected by mutator mutations could display a haplo-insufficiency phenotype (such as mutations affecting a repression function). Secondly, a large number of genes could each be mutable to yield a mutator phenotype (such as mutations affecting the *Tc1* element itself).

Mutator mutants should prove to be an important tool for *C. elegans* molecular genetics. Because *Tc1* insertion causes most mutator-induced mutations, mutant genes can be cloned using 'transposon-tagging' techniques. Mutator mutants have high frequencies of spontaneous mutations affecting many different genes. Spontaneous mutations affecting over 50 visible, developmental, and behavioural genes have been identified from *mut-2(r459)* alone (*C. elegans* workers, personal communications).

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The HIV 'A' (*sor*) gene product is essential for virus infectivity

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The genome of the human immunodeficiency virus (HIV) contains several open reading frames (ORFs) not present in other viruses. The 'A' gene¹, also known as *Q² P³*, *ORF-1⁴* or *sor*⁵, partially overlaps the *pol* gene; its protein product has a relative molecular mass of 23,000 (*M_r* 23K) and is present in productively infected cells⁷⁻¹⁰. The function of this protein is unclear; mutant viruses deleted in 'A' replicate in and kill CD4⁺ lymphocyte lines⁸, but the high degree of conservation of the deduced amino-acid sequence in nine different HIV isolates (80%) and the presence of analogous genes in HIV-2¹¹ and other lentiviruses suggest that the gene

function is an important one. Here we describe a mutant virus deficient in the 'A' gene which produces virion particles normally; however, the particles are ~1,000 times less infective than wild type. Transcomplementation experiments partially restore infectivity. The mutant virus spreads efficiently when virus-producing cells are co-cultivated with CD4⁺ lymphocytes, however, indicating that HIV can spread from cell to cell in a mechanism that does not require the 'A' gene product and probably does not require the production of infective virus particles.

We have created a plasmid, pΔA, containing a mutant 'A' gene by eliminating 620 base pairs (bp) between the *Nde*I site at position 5,158 and the *Eco*RI site at position 5,779 (Fig. 1). This plasmid and the infectious plasmid pNL432 were introduced into SW480 cells, which efficiently express transfected DNA^{12,13}, using the calcium phosphate coprecipitation technique^{14,15}. Virus production could be detected within 24 h (data not shown), and no significant difference in the transient expression of either wild-type or the ΔA mutant plasmid was observed by immunoblotting or by electron microscopy and the reverse transcriptase (RT) activity in the culture fluids were indistinguishable (see Fig. 2, Sup [Day1], for example). These results indicate that the HIV 'A' gene product is not important in the regulation of viral gene activity or in the assembly and release of progeny virions.

Although SW480 colon carcinoma cells can be directly infected with HIV¹⁶, virus propagation is inefficient compared to CD4⁺ lymphocyte cell lines. Thus transfection of SW480 cells measures virus production, not virus infectivity. The latter can be measured, by either cocultivating CD4⁺ lymphocytes with virus-producing SW480 cells or infecting CD4⁺ lymphocytes with cell-free supernatant from transfected SW480 cells. Initially, we used coculture with the CD4⁺ lymphocytic leukaemia line A3.01¹⁷. The ΔA virions could infect A3.01 cells in this system (Fig. 2, top), but unlike pNL432 infection, which peaked between days 7 and 9 and persisted for an additional 10 days, the A3.01 cells continued to produce ΔA virus particles for at least 82 days (data not shown). Neither wild-type nor ΔA progeny could infect cells of the CD4⁻ A2.01 line¹⁸ (data not shown), indicating that expression of the CD4 molecule is required to spread virus by cocultivation.

Cell-free samples of ΔA and wild-type virus particles were prepared from the supernatants of SW480 cells transfected with pΔA and pNL432 by low-speed centrifugation (1,000 g for 10 min) and filtration through a 0.22 μm filter. A3.01 cells were infected with equivalent (RT units) amounts of each virus preparation and HIV replication was monitored (Fig. 2, bottom). Plasmid pNL432 gave a spreading infection detected on day 5 with a peak of RT activity occurring on day 11, but ΔA particles failed to infect the A3.01 cells during the same observation period, indicating that the mutant virions contain an intrinsic defect affecting an early event in the infectious cycle.

We used the pNLA3 plasmid, a derivative of the 1-11 'A' cDNA clone (Fig. 1), to attempt to complement the defect in pΔA. Equal amounts of pΔA and pNLA3 were cotransfected into SW480 cells; the infectious proviral DNA clone pNL432 and pΔA were used as positive and negative controls, respectively. Supernatants were harvested after 24 h, filtered and tested for infectivity on A3.01 cells. Approximately equivalent amounts, measured by RT activity, were used. As shown in Fig. 3, progeny particles were detected by day 8 in cells infected with the filtrate from the pNL432 transfection, but not when ΔA was used. Complementation of the ΔA defect by the pNLA3 clone was observed; virus was detected in infected A3.01 cells by day 19, after which gradually increasing amounts of progeny were synthesized. The slower kinetics observed can be explained if complementation generates infectious particles containing the mutant genome, which can initiate an exogenous infection but, like the ΔA mutant, can only spread via a cell-to-cell mechanism. Because this is not as efficient as infection induced by wild-type particles (see Fig. 2), the infection spreads with delayed kinetics.

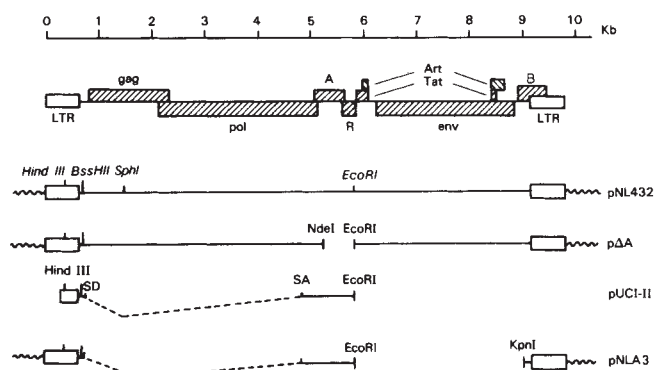


Fig. 1 Structure of HIV clones used to characterize the 'A' gene.

Top, genomic structure of the HIV proviral DNA. The infectious cloned proviral DNA, pNL432 (ref. 12), was initially cleaved with *Nde*I. *Eco*RI linkers were ligated to the digestion products after the 5' overhanging *Nde*I ends were filled in with the Klenow fragment of *E. coli* DNA polymerase I. The mixture was digested with *Sph*I and *Eco*RI generating the 3.7-kb fragment which maps from the *Sph*I site at position 1,443 to the *Nde*I site (containing the *Eco*RI linker) at position 5,158. This fragment was isolated from a preparative agarose gel and inserted into *Sph*I/*Eco*RI-digested pNL432, generating the pΔA plasmid, which contains only the amino-terminal 29 amino-acid residues of the 'A' ORF. This deletion also eliminated 126 bp immediately 3' to the 'A' gene, extending into the region designated R²⁰. An oligo-dT primed λgt10 cDNA library was constructed from lysates of virus-producing H9 cells, which express the 5.5- and 5.0-kb 'A' gene RNAs⁶. One clone (1-11) was obtained containing a 1.1-kb insert which was subcloned into pUC18 [pUC1-11] and M13mp19. The exact structure of the 1-11 cDNA clone was determined by nucleotide sequencing using the Sanger chain-termination method²². The cDNA began 16 nucleotides 3' to the cap site of all HIV mRNAs, extended an additional 273 bp to a splice donor at nucleotide 289, and was joined to nucleotide 4,459 in the *pol* ORF. Clone 1-11 therefore contains a 402 bp 5' untranslated leader sequence that precedes an ATG at nucleotide 4,587; this ATG is followed by an ORF encoding 192 amino acids. The cDNA terminates at the *Eco*RI site, 126 bp 3' to the end of the A ORF. The portion of the cDNA clone located between the *Bss*HII and the *Eco*RI sites was inserted into the infectious molecular plasmid pNL432, thereby placing a complete HIV LTR on both sides of the cDNA and retaining the splice characteristic of the 'A' gene mRNA. The resulting plasmid (pNLA1, not shown) was digested with *Eco*RI and *Kpn*I to eliminate sequences between positions 5,779 and 9,026 encoding *tat*, *art*, *env* and parts of *R* and *B* and religated to generate pNLA3.

We wished to ascertain whether complementation or recombination was responsible for the appearance of progeny particles in these experiments. We prepared DNA by the Hirt procedure¹⁹ from A3.01 cells which had been infected with 'complemented' virus, (pΔA+pNLA3), two weeks after the initial appearance of RT activity. The DNA was digested with *Hind*III and subjected to Southern blot hybridization using the 6.5-kilobase (kb)pBenn6 probe¹⁷ (mapping between positions 1,712 and 8,188). Two fragments of 3.7 and 2.1 kb were found, consistent with the presence of ΔA proviral DNA; wild-type pNL432 proviral DNA gives fragments of 4.4 and 2.1 kb. Further, progeny virus particles, generated in A3.01 cells after inoculation with the 'complemented' filtrate behaved exactly like the ΔA mutant, failing to initiate a spreading infection in the A3.01 cells. Both of these results are compatible with transcomplementation but not recombination.

Higher multiplicities of ΔA virus give detectable infection of CD4⁺ cells but with slow progeny particle synthesis compared to parental virus. The relative infectivity of ΔA particles, as determined by limiting dilution, is ~1,000-fold less than wild type (data not shown). Cell death and syncytia formation in ΔA-infected cells was observed, but at significantly lower levels than for wild-type HIV.

Fig. 2 Effect of the 'A' gene deletion on the infectivity of virus particles. Plasmid DNA (15 µg) from the infectious molecular HIV clone (pNL432) or the 'A' gene deletion proviral DNA (pΔA) were transfected into SW480 cells growing in 25-cm² tissue culture flasks, using the calcium phosphate precipitation procedure^{14,15}. In the cocultivation experiments, 2 × 10⁶ A3.01 cells were added to the SW480 monolayer 24 h after transfection and the cultures were maintained in 5 ml of RPMI1640 medium containing 10% fetal calf serum for 26 days. Infectivity of progeny virions, present in the supernatants of the transfected cells, was evaluated following filtration and determination of RT activity¹³. Comparable amounts (pNL432: 1.1 × 10⁵ c.p.m.; pΔA: 9.2 × 10⁴ c.p.m.) of cell-free virus were used to infect 2 × 10⁶ A3.01 cells propagated in 5 ml of RPMI medium. Aliquots were tested for RT activity at the indicated times. The cell density in all cultures was maintained at ~1 × 10⁶ ml⁻¹.

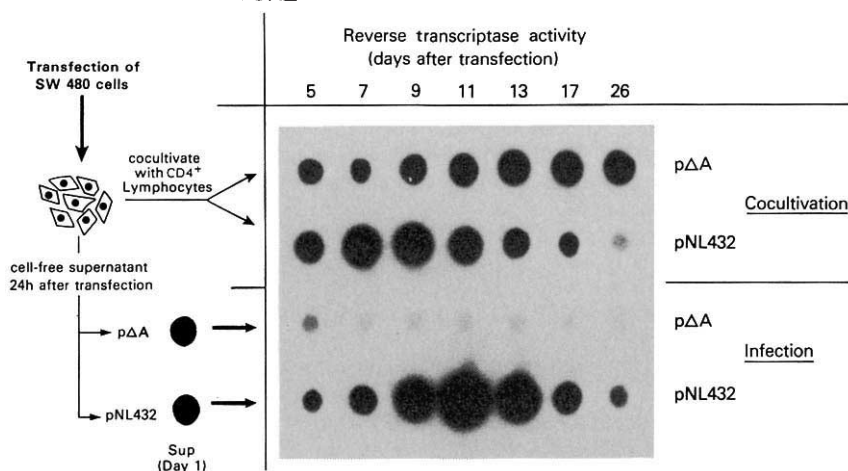
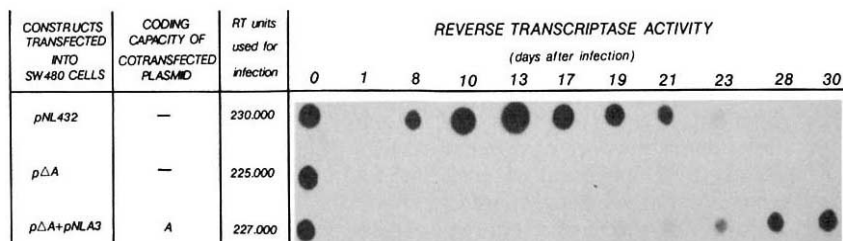


Fig. 3 Complementation of the 'A' gene mutation with the cDNA clone. SW480 cells were transfected with the indicated plasmids (15 µg). In the case of pNL432 and pΔA, the amount of DNA was adjusted to 30 µg by the addition of pUC18. Virus stocks were harvested from SW480 cells 24 h after transfection. Comparable amounts of virus particles, as determined by RT activity, in the cell-free filtrates were added to 2 × 10⁶ A3.01 cells as described in Fig. 2. Cells were cultivated at a density of ~1 × 10⁶ cells ml⁻¹ and aliquots of the culture fluid were tested for RT activity at the indicated times.



As virus lacking the 'A' gene can spread in cocultures, some property of the mutant virions must preclude efficient initiation of productive infection. The fact that replication-competent virus particles can be generated by transcomplementation raises the possibility that the 'A' protein may be required for adsorption, penetration, endocytosis, or uncoating and suggests that it is virion-associated. If the 'A' protein is not an integral component of HIV particles, it could serve a scaffolding function required during packaging. Preliminary results showing that ΔA particles can support an endogenous RT reaction *in vitro* suggest that the mutant virions contain RNA (data not shown).

The existence of the short R ORF in both the HIV and visna virus genomes was recently reported²⁰. As the 620-bp deletion present in pΔA also eliminates 63 codons from the amino terminus of the putative R-gene product, it was important to show that the ΔA phenotype reflected changes only in the HIV 'A' gene. We therefore made a mutant containing an alteration in the R ORF (pNLΔRI) by digesting the infectious HIV clone at the single *EcoRI* site (see Fig. 1), filling in the termini with the Klenow fragment of *E. coli* DNA polymerase I, and ligating the flush ends with T4 DNA ligase. This generated a frame shift that terminates the 96 codon R gene at codon 63. The ΔR particles resulting from the transfection of SW480 cells with pNLΔRI, did not show the defect characteristic of ΔA virions. Furthermore, complementation of pΔA with pNLΔ3 (Fig. 3), which contains only a portion of the R ORF, indicates that the ΔA phenotype is not due to a concomitant deletion in the R gene. Cotransfection of pΔA with pAR(tat)²¹, a plasmid which expresses the HIV tat protein, does not complement the ΔA mutation (data not shown).

The mechanism by which the ΔA infection spreads from cell to cell is presently unclear. As the recipient cells must express the CD4 epitope it is very likely that fusion is involved. HIV structures passed to recipient cells may include replication-competent RNA-protein complexes or unintegrated proviral

DNA. Integration of the latter into chromosomal DNA would sustain a spreading productive infection and avoid many of the early events associated with an exogenous infection.

Cell-to-cell spread of HIV has been suggested as a possible explanation for the chronic nature of the viral infection *in vivo* which persists despite the antibody response. We have shown here that for the ΔA mutant this is the principal mechanism of virus spread. Although not formally demonstrated, it must be assumed that wild-type HIV can also spread cell-to-cell. This result has implications for the epidemiology of HIV infection as well as strategies of antiviral and vaccine intervention.

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Generation of an antibody with enhanced affinity and specificity for its antigen by protein engineering

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A detailed description of the interactions between an antibody and its epitope is necessary to allow an understanding of the way in which antibodies bind to antigenic surfaces presented by foreign molecules. Ideally this should be done by analysis of crystal structures of antibody-antigen complexes, but so far only two of these are available^{1,2}. An alternative strategy³ combines molecular modelling⁴⁻⁶ with site-directed mutagenesis (SDM) and using this we have generated a preliminary model⁷ of the complex between Gloop2, an antibody raised against a peptide containing the 'loop' determinant of hen egg-white lysozyme (HEL) which also binds the native protein⁸, and its epitope on the protein surface. The main predictions from our model were; (1) that the surface of interaction between the antibody and the antigen is large ($20 \text{ \AA} \times 15 \text{ \AA}$) and involves all the complementarity-determining regions (CDRs), (2) that electrostatic interactions were important in the formation of the complex, and (3) that conformational changes in either the loop or in the CDRs may occur during the formation of the complex. Here we report SDM studies which test some of these predictions; removal of two charged residues at the periphery of the combining site increases the affinity of the antibody for its antigen over 8-fold and decreases its ability to cross-react with closely-related antigens. This result is at variance with our original prediction but can be accommodated within our newly refined model; the role of electrostatics in antigen-antibody interactions is now questionable.

The antibody-combining site is formed by the juxtaposition of six hypervariable or CDRs, three deriving from the light chain (designated L1, L2 and L3) and three from the heavy chain (H1, H2 and H3). Although the structural details of a number of antibody-combining sites are known⁹⁻¹⁴, the manner in which

CDR-backbone length and sequence influences the final topology is unknown. Such information may eventually be derived from comparisons within a larger data base of structures than presently exists.

Our initial analysis of the computer model of the Gloop2-HEL complex, together with the results of binding studies of Gloop2 and a panel of variant avian lysozymes⁸, strongly implicated the interaction of (1) Glu 28 (27A using the Kabat numbering system¹⁵), in the light chain CDR1 (L1), with Arg 68 (HEL) and (2) Lys 56, in the heavy chain CDR2 (H2), with Asn 77 (HEL). In neither case are the residue pairs close enough to form hydrogen bonds (closest contact $4.7 \text{ \AA}$), but it was suggested that they may be important in the orientation of the two interacting protein surfaces. Based on these initial observations both Glu 28 (L1) and Lys 56 (H2) were chosen as candidates for mutagenesis. There were also a number of residues that appeared to be partially buried within the combining site, inaccessible to antigen contact and yet variable between the individual antibodies. Glu 50 in the heavy chain H2 of Gloop2 fell into this category. Four types of substitution were therefore made by SDM: Glu 28 to Ser, Lys 56 to Gln and Glu 50 to Ser and the double mutant Glu 28 to Ser: Lys 56 to Gln according to the scheme shown in Fig. 1, and the mutant proteins were expressed¹⁶.

In parallel with the mutagenesis studies, further refinement of the initial model for the Gloop2-HEL complex was carried out by energy minimization, using the GROMOS molecular dynamics package¹⁷. Regions of unacceptable stereochemistry in the centre of the combining site region (Fig. 2a) were completely eliminated from the model by this procedure, in addition to a general enhancement of the complementarity of the antibody and antigen surfaces (Figure 2b). The principal contact residues, however, remain unchanged between the old and new models. The energy minimization of the complex showed that only minor conformational changes ($<0.5 \text{ \AA}$) would be required within the antibody and the antigen for the two molecules to dock together to form a complex. This is consistent with observations from the experimental study of Poljak and co-workers¹.

The results of binding assays performed with the mutant antibodies, using HEL and Pep1 (a loop-containing peptide) as antigens, are shown in Fig. 3a and summarized in Table 1. The single mutant Glu 28 to Ser showed a moderate increase in

Fig. 1 a, The cloning of Gloop2 heavy and light chain complementary DNAs and their expression by *in vitro* transcription with SP6, and *in vivo* translation with *Xenopus* oocytes to generate fully assembled and functional antibodies has been described elsewhere¹⁶. The same procedure was used to express the mutant antibodies and SDM was performed using the *EcoK/EcoB* reciprocating selection system¹⁹. The cDNA clones were subcloned in an inverted orientation into the M13 mutagenesis vector (M13K19) such that the mutagenic primers were 5' to the selection primers. Mutagenesis using the mutagenic primer, MUTL28, converted Glu 28(GAA) in CDR1 of the light chain to Ser (AGT) by three mismatches (*), the fourth being silent. The mutagenic primers MUTH50 and MUTH56 generated changes within the CDR2 of the heavy chain at position 50, converting a Glu (GAA) to a Ser (TCA) by two mismatches, and at position 56, a Lys (AAG) to a Gln (CAG) by one mismatch. b, Mutant RNA transcripts were synthesized by subcloning the mutated cDNA clones as *Bam*HI fragments into the *Bgl*II or *Bgl*III/*Bam*HI-restricted vector, pSP64T. All constructs were linearized by *Eco*R1 restriction. The single mutant antibodies, E28S, E50S and K56Q were prepared by microinjection of the mutant RNA transcript with the appropriate 'wild-type' heavy or light chain RNA transcript into the cytoplasm of *Xenopus* oocytes; the double mutant E28SK56Q was produced by microinjection of the two mutant transcripts of E28S and K56Q. SS, signal peptide sequence; V, variable region and C, constant region of either the light (κ) or γ 2b heavy chain (H); UT, untranslated region; SP6, promoter region from *Salmonella typhimurium* phage; X-UT, the 5' and 3' UT regions of the *Xenopus* β -globin gene; PL, polylinker.

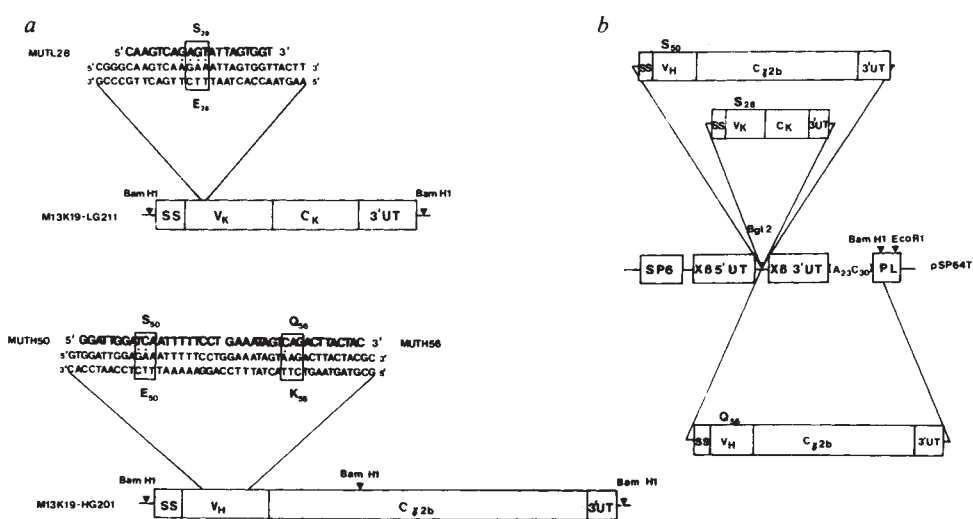


Fig. 2 Combining site region of the Gloop2/HEL complex⁷ before (a) and after (b) refinement of the model by energy minimization. In a, note the unacceptably close contacts between (1) main-chain atoms in the region of Leu 92 and Ser 93 of L3, and (2) side-chain atoms in the region of Tyr 32(L1) and Pro 70(HEL). In panel b, these bad contact regions are no longer present and the complementarity of the two molecular surfaces is clearly improved. The improvement in the model resulting from the energy minimization was reflected in the large potential energy (p.e.) drop over the 164 cycles of steepest descent minimization: p.e. (initial model) = $+2.95 \times 10^{11}$ kcal mol⁻¹; p.e. (minimized model) = -0.533×10^4 kcal mol⁻¹. A van der Waals representation of the antibody (blue): antigen (green) surfaces (c shows model a, and d shows model b) indicates the general stereochemical complementarity of the two molecules in the region of the combining site. Hydrogen bonds, both within the individual molecules themselves, and between antigen and antibody, are indicated (---).

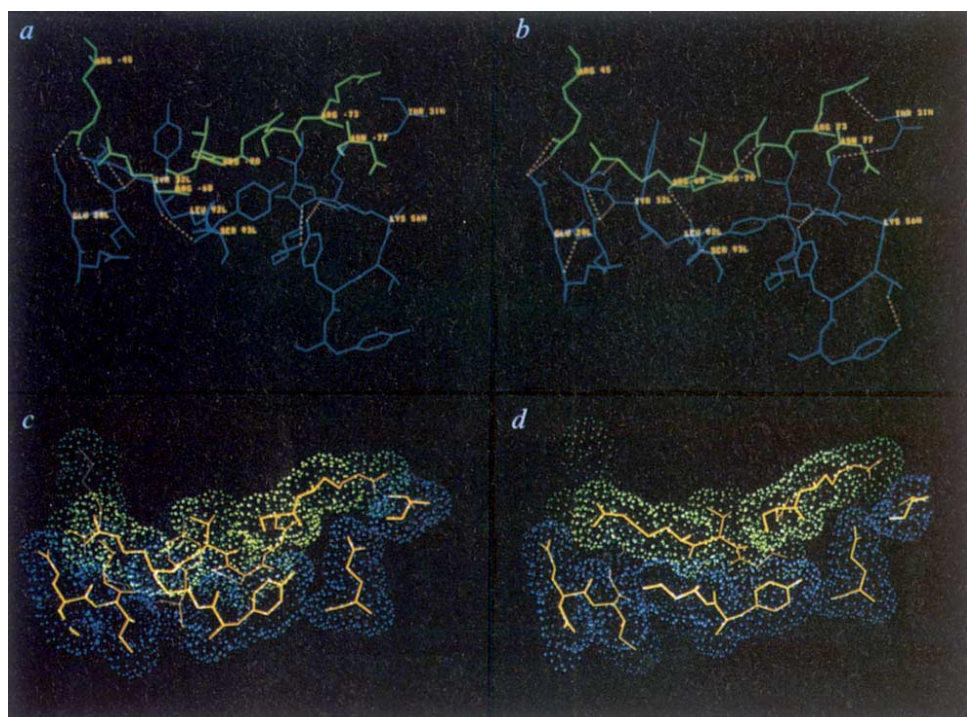


Table 1 Affinity constants (k_D/M) for binding of Gloop 2 and its mutants to Pep1 and HEL

	Gloop 2	E28S	K56Q	E28SK56Q
Pep1	$2.0 \pm 0.75 \times 10^{-8}$	8.2×10^{-9}	2.0×10^{-8}	4.8×10^{-9}
HEL	$2.3 \pm 1.0 \times 10^{-7}$	4.7×10^{-8}	$1.9 \pm 0.12 \times 10^{-7}$	$2.75 \pm 0.02 \times 10^{-8}$

Assays were carried out in triplicate as described in Roberts and Rees¹⁶. Essentially microtitre plates were coated with goat anti-mouse IgG antibody (affinity purified) at a concentration of $50 \mu\text{g ml}^{-1}$ in PBS for 18 h at 4°C. After coating the plates were blocked with PBS containing 0.05% Tween for 30 min at 4°C, then 2 × 1 min at room temperature. Oocyte test supernatant was added and incubated at room temperature for 6 h. Plates were washed and ¹²⁵I-labelled Pep1 with and without inhibitor was added and incubated at room temperature for a further 6 h. Plates were washed with PBS/Tween 3 × 1 min and individual wells cut out and counted. The k_D values for Pep1 inhibition of ¹²⁵I-labelled Pep1 binding were obtained from Scatchard analysis. As Scatchard analysis was inappropriate for analysis of HEL inhibition of Pep1 binding, k_D values were obtained by the following method. Using the program SANCOL (R. Ryan, unpublished) a non-linear regression procedure²² was used to fit the binding equation which relates specifically bound units (for instance c.p.m.—the measured variable) and the total ligand concentration (the control variable) to the experimental data²³. Scatchard estimates of the k_D and total site concentration (S_T) values were then used as a start point for an iterative procedure which calculated the final k_D and S_T values. Binding data were measured at pH 7.2. The range of means obtained in different experiments is given.

affinity for both Pep1 and HEL (3–4 fold) whereas the mutant Lys 56 to Gln showed no significant change in binding. But combining the two single mutations within the same antibody gave a double mutant which showed a marked increase in affinity for HEL (8–9-fold), and a moderate increase for Pep1 (4–5-fold) (Fig. 3a).

When this analysis was extended to the variant lysozymes (Table 2) the pattern of binding satisfied the purely thermodynamic criteria that, if the mutations improved the complementarity of Gloop2 for its native antigen HEL, then the variant lysozyme with the highest affinity, but non-identical complementarity, for wild type Gloop2 should experience the most drastic loss of affinity (6–7-fold decrease in relative affinity for TEL, Table 2). By contrast, those variant lysozymes with somewhat lower affinities, and hence rather less stringent interactions, would be less affected (2–4-fold decrease in relative affinity for BWQEL, RNPEL and HuL, Table 2). The double mutant thus

Table 2 Relative affinities of Gloop 2 and its mutants for lysozymes of different species

	Gloop 2	E28S	K56Q	E28SK56Q
TEL	0.5	3.0	0.5	7
BWQEL	124	111	ND	235
RNPEL	13	ND	12	30
HuL	370	ND	ND	1300

The numbers shown express ID₅₀ values for inhibition of binding of ¹²⁵I-labelled Pep 1 to antibody by the appropriate variant lysozyme (methods as described in the legend to Table 1). Each value has been normalized to the ID₅₀ value for binding of the appropriate antibody to HEL. Variant lysozymes used in this study are: TEL, turkey egg lysozyme; BWQEL, bobwhite quail egg lysozyme; RNPEL, ring-necked pheasant egg lysozyme, and HuL, human lysozyme.

showed not only a greater affinity for HEL, but also an increased specificity towards HEL over the other avian species. Modelling of these amino-acid changes in the antibody L1 and H2 CDRs suggests that the removal of the electrostatic residues, and their replacement by non-charged hydrophilic residues, allows a closer fit of antibody and antigen. As a consequence there is a general improvement in hydrogen bonding between the two surfaces, but without significant changes in the interactions within the antibody CDR's at the sites of the mutations (Fig. 3b).

By contrast, mutation of the partially-buried Glu 50 in H2 resulted in abolition of binding between the antibody and HEL or Pep1 (Fig. 3c). The residue in this position is the first to emerge from the framework region preceding CDR H2 and shows a high variability between heavy chains of the same V_HII subgroup¹⁵. In the model this residue appears to be both hydrogen bonded to Tyr 94 in neighbouring CDR L3 and involved in a salt-bridge-like interaction with Arg 101 of H3. Substitution at this position would be predicted to cause a significant change in the relative conformations of adjacent, interacting CDR's. Such perturbations, if they occurred, could more than account for the observed loss of binding. This observation may be an example of a situation where the existence of variability between antibodies at a particular residue position does not necessarily signify a minor structural role for that position in the combining

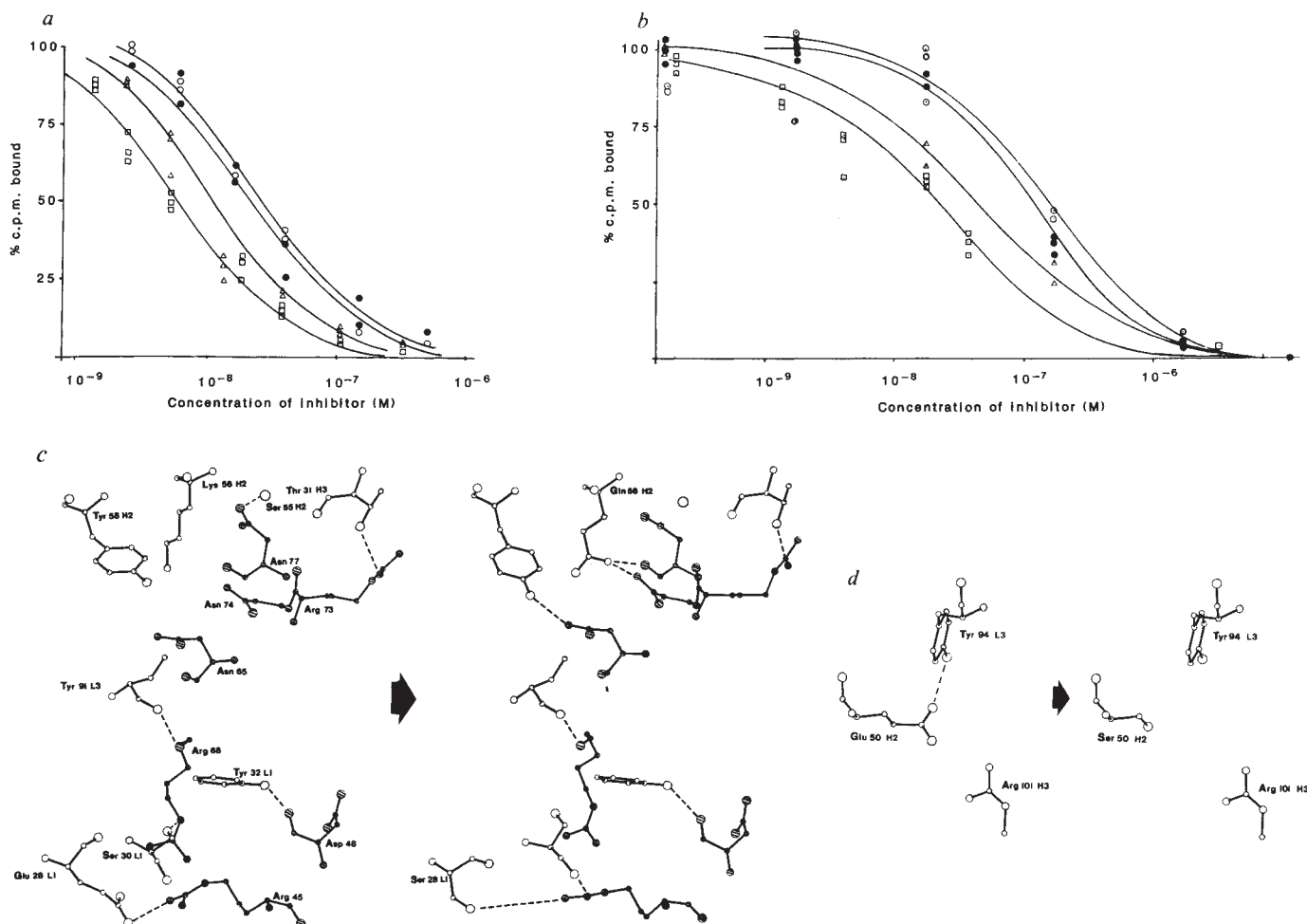


Fig. 3 Inhibition of binding of ^{125}I -labelled Pep1 to Gloop2 (○) and mutant antibodies, k56Q (●), E28S (△) and E28S K56Q (□) by *a*, Pep1 and *b*, HEL. *c*, Predicted effect of the combined mutations Glu 28-Ser (L1) and Lys 56-Gln, in the region of the antibody combining site. The modelling would suggest that hydrogen bonding interactions (---) within the antibody CDR would be preserved, and in addition interactions with the antigen enhanced; Asn 65(HEL) → Tyr 58(H2), Asn 74(HEL) → Gln 56(H2), Asn 77(HEL) → Gln 56(H2), Arg 45(HEL) → Ser 28(L1) and Arg 45(HEL) → Ser 30(L1) all appear as new contacts. *d*, Predicted effect of the mutation Glu 50 → Ser (H2). Here the modelling suggests the replacement of the glutamic-acid residue by a serine will have a significant effect on the nature of the antibody-combining site, with the loss of two important interactions: (1) a hydrogen bond between Tyr 94 OH(L3) and Glu 50 OE1 (H2) and (2) a salt-bridge-like interaction between Glu 50 (H2) and Arg 101(H3). Amino-acid changes were incorporated into the model by introducing the new side chains with FRODO²⁰ on the PS300, and then energy minimizing the entire structure, using GROMOS¹⁷, to obtain a model for the mutant protein²¹.

site. Different combinations of CDR may have requirements for specific inter-CDR interactions. Thus, when assessing the structural consequences of somatic mutations *in vivo* or *in vitro*, both antigen-antibody and CDR-CDR interactions should be considered.

These preliminary results therefore raise two important questions concerning antibody-antigen interactions, and suggest a number of possible mutations for further study. Our original premise was that the two charged groups lying at opposite edges of the combining site (Glu 28 and Lys 56) were important contacts in the antigen interaction and, further, might actually play a role in orientating the loop region of HEL. But the engineering of an antibody with enhanced affinity for its antigen by the removal of these proposed 'key' electrostatic residues, and their replacement by non-charged polar residues, questions this hypothesis. Our results would indicate that the hydrogen-bonding between the two molecules over the combining site surface is of paramount importance. The electrostatic residue in the antigen (Arg 68), no longer paired across the interface after mutagenesis, is easily accessible to solvation by water molecules. In support, recent binding data show: (1) there is no difference in the k_{on} values for the binding of Pep1 to Gloop2

over the ionic strength range 0.01 M to 0.5 M, such as might be expected if electrostatic orientation was important in acquisition of the complex, (2) the k_{D} for binding of Pep1 to Gloop2 actually decreases with ionic strength from 1.15×10^{-8} M (at 0.01 M) to 3.8×10^{-9} M (at 0.50 M). As k_{on} is unchanged this result indicates that the charged residues actually exert an inhibitory effect on binding, and that when their effective charge is screened improved protein-protein contacts are possible. This proposition is consistent with the mutation experiment where the removal of the charges results in increased affinity. The possible counter-argument that, by substitution of two charged residues the surface has been rendered more 'sticky' by altering its hydrophobic/hydrophilic character is not tenable because solvent accessibility calculations¹⁸ suggest that the hydrophilic character is largely unchanged by the substitutions Lys 56 to Gln and Glu 28 to Ser ($\text{SA} < 30 \text{ \AA}^2$).

In conclusion, we have demonstrated that it is possible to engineer an anti-peptide antibody in such a way that its affinity for the same epitope in the native protein is increased and, concomitantly, its cross-reactivity with related antigens is reduced. Inspection of the refined model suggests additional mutations that may lead to further improvements in affinity.

This approach offers a possible solution to the problem of how to generate high affinity antibodies against intact antigens when peptides are used as immunogens and thus has far-reaching therapeutic consequences.

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Interaction of an embryo DNA binding protein with a soybean lectin gene upstream region

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Seed protein genes are highly regulated during the soybean life cycle^{1,2}. These genes encode prevalent mRNAs that accumulate and decay during embryogenesis, and are either undetectable or present at low levels in mature plant organ systems^{2,3}. Transcriptional activation and repression processes are important in regulating seed protein gene developmental expression programs^{1,2}. We started DNA binding protein studies with the soybean lectin gene⁴⁻⁶ to begin to identify *trans*-acting proteins and *cis*-regulatory sequences required for seed protein gene expression. We have identified an embryo DNA binding protein that interacts with specific sequences in the lectin gene 5' region. The DNA binding protein is undetectable in mature plant organ systems and its concentration parallels the lectin gene transcription rate during embryogenesis. The DNA binding protein activity corresponds to a 60,000 M_r (60K) nuclear protein, and a protein of similar size interacts with at least one other seed protein gene but not with a gene inactive during embryogenesis. Our data suggest that the 60K protein, and the DNA sequences that it interacts with, may be involved in regulating lectin gene expression.

Figure 1a schematically shows the lectin gene region. We isolated this region as a 17.1-kilobase (kb) *Eco*RI fragment from

a λ Charon 4 soybean genomic library^{4,7}. In addition to the lectin gene, the 17.1-kb fragment contains at least four nonseed protein genes⁶. Figure 1b shows the lectin gene and relevant reference sequences⁵. The lectin gene is intronless, encodes a 1.1-kb mRNA, and is expressed during specific embryonic periods and in the mature plant root⁶. *In situ* hybridization studies showed that lectin mRNA is represented primarily in embryo cotyledon cells and in root ground meristem tissue (L. Perez-Grau and R.B.G., unpublished data). By contrast, the nonseed protein genes are expressed throughout embryogenesis and in mature plant leaf, root, and stem cells⁶. Thus the lectin gene is regulated temporally and spatially during the soybean life cycle, and is embedded in a domain with several differentially expressed genes.

We isolated nuclear proteins from mid-maturation stage embryos, 75 days after flowering (DAF)⁸ and then reacted these proteins with lectin gene fragments to identify *cis*-elements and *trans*-factors that are involved in regulating lectin gene expression. At mid-maturation, cell division has ceased, embryo cells are expanding and accumulating seed proteins, and lectin mRNA is ~0.75% of the embryo mRNA mass^{4,8}. Figure 1c shows the results of a DNA gel electrophoresis mobility retardation assay⁹ using a lectin gene 5' probe (Lel 5', Fig. 1b). We used this probe initially because gene transfer studies showed that the lectin gene is regulated correctly in tobacco⁶, and that 0.50 kb of 5' sequence (pLelΔ0.5, Fig. 1a) is sufficient to program its expression during seed development (J.K.O. and R.B.G., unpublished results). As seen in Fig. 1c, Lel 5' probe mobility was retarded significantly in the presence of nuclear proteins indicating that protein-DNA complexes formed (lanes NP and O). Addition of unlabelled poly(dI-dC)·poly(dI-dC) duplex DNA to eliminate nonspecific protein-DNA interactions⁹ yielded a free Lel 5' fragment (circle, Fig. 1c) and a more slowly migrating protein-DNA complex (arrow, Fig. 1c). Figure 1d shows that we were unable to detect a protein-DNA complex with a lectin gene 3' probe (Lel 3', Fig. 1b). Nor was a complex detected with a gene that is not expressed in soybean embryos (leghaemoglobin 5', Fig. 1d). We added unlabelled lectin DNA (pH4.4, Fig. 1a), as well as unlabelled pBR322 and *Drosophila* blastoderm gene DNAs, to determine if the protein-DNA complex was specific for the lectin gene. Only the unlabelled lectin DNA eliminated the protein-DNA complex formation (Fig. 1e). Together, these data show that embryo nuclear protein forms a specific complex with the lectin gene, and that the protein-DNA interaction occurs in a 5' gene region.

We reacted the Lel 5' probe (Fig. 1b) with nuclear proteins from embryos at different developmental stages, and from mature plant organ systems, to test whether the DNA binding protein activity correlated with lectin gene transcription levels. The mobility retardation assay in Fig. 2a shows that a protein-DNA complex (arrow) was obtained with the nuclear protein extract of 25-DAF embryos (lane DAF 25) demonstrating that lectin DNA binding protein activity is present early in embryogenesis. We also obtained a protein-DNA complex with similar electrophoretic mobility using the nuclear protein extract from 40-DAF embryos (Fig. 2a, lane DAF 40); however, the proportion of Lel 5' probe in the complex increased significantly. By contrast, the fraction of Lel 5' probe in the protein-DNA complex decreased with the 75-DAF embryo nuclear protein extract (Fig. 2a, lane DAF 75) and was reduced to an undetectable level with nuclear proteins from embryos in the terminal stages of development (Fig. 2a, lane DAF 100). In addition, we were unable to detect a protein-DNA complex with leaf, stem, and root nuclear proteins (Fig. 2a, lanes L, S, R).

We presented elsewhere the relative lectin gene transcription rates and mRNA levels during different stages of the soybean life cycle^{2,6}. Lectin mRNA accumulates and decays during embryogenesis and is undetectable in mature plant leaf and stem. Root lectin mRNA is 10⁴-fold less prevalent than embryo lectin mRNA at mid-embryogenesis (40-75 DAF). Runoff tran-

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Fig. 1 Lectin DNA binding activity in soybean embryos. *a*, The soybean seed lectin gene region. The organization and expression of the lectin gene region were described elsewhere^{4,6}. RI, H3, HI, KI, and XI refer to *Eco*RI, *Hind*III, *Hpa*I, *Kpn*I and *Xba*I restriction endonucleases, respectively. Boxes and arrows approximate gene locations and transcriptional orientations, respectively. The plasmid pH4.4 contains nucleotides -3,540 to +861 relative to the lectin gene transcription start site^{4,6}. The plasmid pLe1Δ0.5 contains the lectin gene and 0.52 kb of 5' upstream sequences. *b*, A schematic representation of the soybean seed lectin gene. Nucleotides +1 and +1,014 mark the 5' and 3' gene ends, respectively^{4,6}. CACA represents

the 5' $\uparrow$ CACA CACA $\uparrow$ 3' consensus sequence that is present in the 5' flanking region of all seed protein genes sequenced to date³. The physiological significance of this sequence, if any, is not yet known. The bracketed lines below the lectin gene represent DNA probes used in the protein-DNA binding reactions and were obtained from plasmid pH4.4. Le1 5' and 3' probes contained sequences -334 and +520 to +861 relative to the transcription start site, respectively⁵. *c*, Interaction of the seed lectin gene with embryo nuclear proteins. NP, control reaction without proteins; 'μg poly dI-dC', reactions containing nuclear proteins and the indicated amounts of poly(dI-dC)·(dI-dC)⁹. Arrow, embryo protein/lectin DNA complex; circle, free DNA fragment. *d*, Embryo nuclear protein interaction with lectin 5' and 3' regions, and the leghaemoglobin 5' gene region. Le1 5' and Le1 3', bracketed regions shown in *b*; + and -, reactions with or without proteins, respectively. Leghaemoglobin 5', a probe that contained sequences -910 to +720 relative to the soybean *Lbc3* leghaemoglobin gene transcription start site¹². This probe was obtained from the λLB11 recombinant phage provided by Dr D. P. S. Verma¹². The *Lbc3* leghaemoglobin gene is expressed exclusively in root nodules¹³. *e*, Influence of unlabelled homologous and heterologous DNAs on lectin DNA-protein complex formation. 'dI-dC' refers to a reaction that contained 4 μg poly(dI-dC)·(dI-dC). Le1: pBR322 and pBR322 refer to unlabelled plasmid pH4.4 DNA (Fig. 1*a*) and unlabelled pBR322 DNA, respectively; *Drosophila* bsg75C' refers to unlabelled DNA from plasmid pLRH31 that contains a *Drosophila* blastoderm-specific gene (R. Baldarelli and J. A. Lengyel), '1:10, 1:20, 1:40 and 1:80' refer to the molar ratio of labelled to unlabelled DNAs in the binding reaction. We estimated the relative affinity ratio of the lectin DNA binding protein for lectin and pBR322 DNAs using the relationship of Singh *et al.*⁹. The fraction of lectin DNA in protein-DNA complex was decreased by 50% with the addition of 1,000 ng of pBR322 DNA and 2 ng of lectin DNA (data not shown). Assuming that pBR322 (4,362 bp) has 4,362 non-specific sites⁹ and knowing that lectin 5' DNA has two high-affinity sites (Fig. 4), we calculated that the pBR322/Le1 5' embryo binding protein affinity ratio is [(1,000 ng)/(2 ng)] × [(4,362 sites)/(2 sites)] or ~10⁶. This is a minimum estimate because the relationship⁹ does not take into account the number of non-specific affinity sites associated with the homologous DNA.

Methods. Soybean embryos were harvested and staged as previously outlined^{1,8}. Nuclei were isolated according to Walling *et al.*² and proteins were extracted by the procedure of Miskimins *et al.*¹⁰. A typical nuclear preparation contained ~5 × 10⁷ nuclei. Unless specified differently, protein-DNA binding reactions were carried out by mixing 0.5 ng (1,000 c.p.m.) of labelled fragment with 8 μg of soybean 75 DAF embryo nuclear proteins and 4 μg of poly(dI-dC)·(dI-dC) in 25 μl of binding buffer (10 mM Tris pH 7.5, 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 5% glycerol⁹). Reactions were carried out for 30 min at room temperature. The free DNA and DNA-protein complex were separated by agarose gel electrophoresis in a low-ionic strength buffer with circulation⁹. Following electrophoresis, the gel was dried and exposed to X-ray film with an intensifying screen at -70 °C for 12-24 h.

scription studies with isolated nuclei showed that relative lectin gene transcription rates parallel lectin mRNA levels, indicating that lectin gene expression is controlled in part at the transcriptional level². Lectin gene transcription rates correlate well with the presence of lectin DNA binding protein activity (Fig. 2*a* and *b*). For example, 40-DAF embryos had maximum DNA binding protein activity and the highest relative lectin gene transcription rate (Fig. 2*a* and *b*, lane DAF 40). Together, these findings indicate that lectin DNA binding protein activity is developmentally regulated, and that there is a direct relationship between lectin gene transcription and DNA binding protein levels.

We reacted embryo nuclear protein gel blots¹⁰ with a lectin DNA probe (pLe1Δ0.5, Fig. 1*a*) to determine if we could detect the protein responsible for the DNA binding activity in Figs 1 and 2. Figure 3*A* shows the stained nuclear protein profiles for 40-DAF and 75-DAF embryos, as well as for the mature plant stem. An identical spectrum of prevalent nuclear proteins was detected at each developmental stage and no quantitative differences were observed. In addition, the nuclear protein profile was distinct from that observed with total embryo protein (data not shown). As seen in Fig. 3*b* the lectin gene probe interacted with several embryo nuclear proteins. Most of the DNA binding proteins persisted throughout embryogenesis, bound to nonlectin DNA probes (Fig. 3*c* and *d*), and decreased continuously with developmental time (compare lanes 25 DAF to 100 DAF, Fig. 3*b-d*). By contrast, the lectin gene probe bound to a 60K nuclear protein (arrow, Fig. 3*b*) that exhibited a developmentally regulated programme similar to that observed for the protein-DNA complex shown in Fig. 2*a*. The 60K protein increased to a maximum level in 40-DAF embryos (lane 40, DAF, Fig. 3*b*) and then decreased to an undetectable level before seed dormancy (lane 100 DAF, Fig. 3*b*).

We also reacted embryo nuclear protein gel blots with a soybean Kunitz trypsin inhibitor gene probe to test whether the 60K DNA binding protein interacted with other seed protein genes. As a control, we reacted the protein gel blots with a

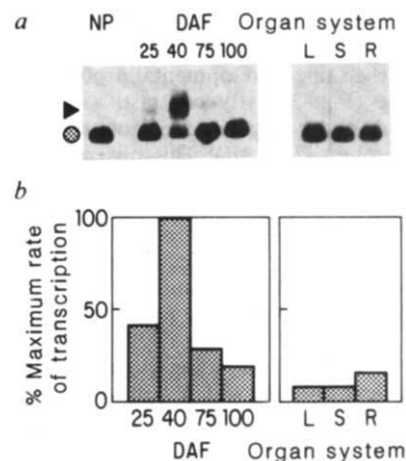
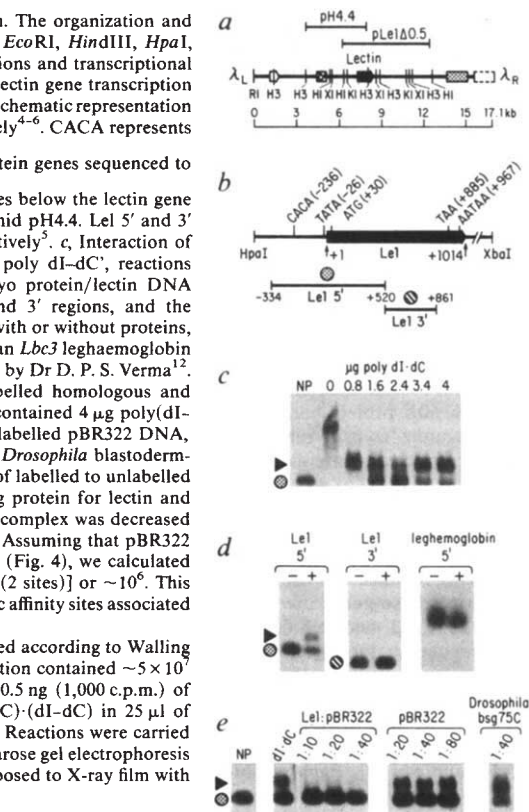


Fig. 2 Lectin DNA binding activity during the soybean life cycle.

a, Interaction of lectin DNA with nuclear proteins from embryos at different developmental stages and from mature plant organ systems. Protein-DNA binding reactions used the Le1 5' probe shown in Fig. 1*b*. NP, reaction without nuclear proteins. DAF 25, 40, 75, and 100, reactions that contained nuclear proteins from 25-, 40-, 75-, and 100-DAF embryos, respectively. L, S, and R, reactions that contained leaf, stem, and root nuclear proteins, respectively. Arrow, protein/lectin DNA complex; circle, free DNA fragment. *b*, relative lectin gene transcription rates during the soybean life cycle. Relative transcription rates were taken from Walling *et al.*² and were obtained from *in vitro* transcription studies with the same nuclei that were used to isolate nuclear proteins.

Methods. Nuclear protein extracts were prepared from embryos of distinct developmental stages and from mature plant leaf, stem, and root (see Fig. 1). Each protein-DNA binding reaction contained the same amount of nuclear protein and was carried out as in Fig. 1.

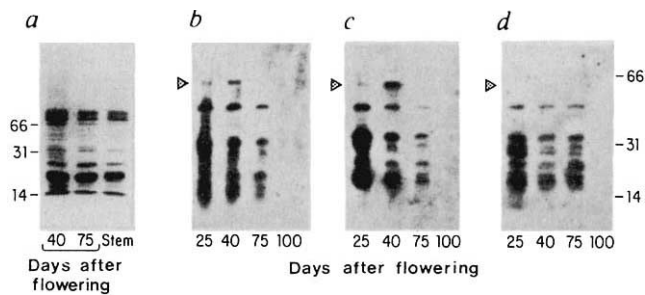


Fig. 3 Reaction of labelled DNA fragments with nuclear protein gel blots. Size markers, M_r in thousands. Lanes 25, 40, 75, 100, and S, contained 25-DAF embryo, 40-DAF embryo, 75-DAF embryo, 100-DAF embryo, and stem nuclear proteins, respectively. Arrow, 60K protein band that interacts specifically with the lectin and Kunitz trypsin inhibitor gene regions. *a*, Coomassie blue stained nuclear proteins. *b*, Reaction with the lectin gene. Plasmid pLe1Δ0.5 (Fig. 1*a*) was used as a probe for the lectin gel blot. The Le1 5' probe (Fig. 1*b*) gave identical results. *c*, Reaction with Kunitz trypsin inhibitor genes. A probe containing two Kunitz trypsin inhibitor genes, designated *KTi1* and *KTi2*, and their 5' and 3' flanking regions, was used for the Kunitz trypsin inhibitor gel blot. Both of these genes are expressed during embryogenesis (refs 1 and 2; K.D.J. and R.B.G., unpublished results). *d*, Reaction with the leghaemoglobin gene. The leghaemoglobin 5' gene probe (Fig. 1*d*) was used for the leghaemoglobin gel blot.

Methods. Gel blots containing nuclear proteins from different embryonic stages were reacted with labelled DNAs as described by Miskimins *et al.*¹⁰ with minor modifications (R. Deans, personal communication). Each gel blot lane contained 20 μ g of nuclear protein.

leghaemoglobin gene probe. We showed previously that Kunitz trypsin inhibitor genes are also temporally and spatially regulated during embryogenesis, and are controlled in part at the transcriptional level^{1,2}. As shown in Fig. 3*c* (arrow), the Kunitz trypsin inhibitor probe interacted with the 60K nuclear protein and produced the same developmental profile as observed with the lectin gene (Fig. 3*b*). By contrast, a gene which is not expressed during embryogenesis does not bind detectably to the 60K nuclear protein (Fig. 3*d*). This observation is consistent with the DNA gel retardation results shown in Fig. 1*d* using the leghaemoglobin 5' probe. Together, these findings suggest that the 60K embryo nuclear protein is responsible for the DNA binding protein activity observed with the DNA mobility retardation assay (Figs 1 and 2), and that this protein recognizes sequences shared by the lectin and Kunitz trypsin inhibitor gene regions.

We localized the DNA binding region more precisely by interacting nuclear proteins with a series of 5' deletions as well as with specific DNA fragments. As shown in Fig. 4*a* and *b*, and summarized in Fig. 4*c*, a protein-DNA complex was not detected using DNA fragments containing sequences -217 to -522 relative to the transcription start site (Fig. 4*b*, lanes 5 and 6). Similarly, no protein-DNA complex was observed using fragments that contained sequences +212 to -77 (Fig. 4*a*, lanes 3 and 4; and Fig. 4*b*, lane 8). These findings, and those in Fig. 1*d*, indicate that there are no detectable embryo DNA binding protein sites in the lectin gene itself, or within two upstream regions (+1 to -77 and -217 to -522). By contrast, Fig. 4*a* (lanes 1 and 2) and Fig. 4*b* (lane 7) show that DNA fragments containing sequences -77 to -217 were able to form an embryo protein-DNA complex indicating that one or more protein binding sites lie within this region.

We carried out a DNase I footprint experiment¹¹ to identify sequences in the -77 to -217 region that recognized the embryo DNA binding protein. For this experiment we used a 0.32-kb DNA fragment containing sequences -334 to -12 relative to the lectin gene transcription start site (Le1D, Fig. 4*c*). Figure 4*d* shows that two regions, designated I and II, were protected

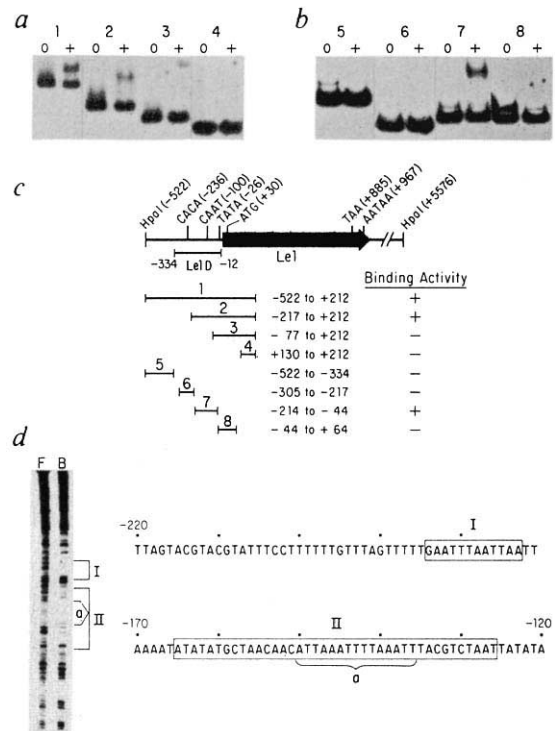


Fig. 4 Lectin DNA sequences that interact with embryo nuclear protein. *a*, Interaction of embryo nuclear protein with lectin 5' deletions. Deletions of sequences 5' to the lectin gene were generated using *Bal31* nuclease¹⁴ and were inserted into the pGEM-1 plasmid¹⁵. The 5' endpoint positions were determined by DNA sequence analysis¹⁶ with reference to the published lectin gene sequence⁵. The relevant labelled DNA fragments were gel-purified and used in protein-DNA binding reactions as in Fig. 1. Reactions 1-4 used probes 1, 2, 3, and 4 (shown schematically in *c*): + and 0, reactions with or without nuclear proteins, respectively. *b*, Interaction of embryo nuclear protein with 5' lectin gene fragments. Labelled DNA fragments representing specific 5' regions were gel-purified from plasmid pH4.4 (Fig. 1*a*) and were used in protein-DNA binding reactions. A schematic representation of the seed lectin gene region is shown for reference (Fig. 1*b*). The 5' and 3' probe termini are designated relative to the transcription start site⁵. Binding activity, represented by + or -, summarizes the gel retardation results shown in *a* and *b*. *c*, DNase I footprint analysis of DNA sequences that interact with embryo nuclear protein. Lane B: the Le1D probe (shown in *c*) containing sequences -334 to -12 relative to the lectin gene transcription start site⁵ was reacted with soybean embryo nuclear proteins as in Fig. 1. Lane F: a parallel reaction was also carried out without nuclear proteins. The reactions were treated with 0.5 μ g ml⁻¹ DNase I in 5 mM MgCl₂ and CaCl₂ for 15 min and were then fractionated by electrophoresis⁹. DNA fragments were visualized by autoradiography, purified¹⁷, and then analysed on a DNA sequencing gel in the presence of 8 M urea. Brackets I, II, and IIA, regions protected from DNase I digestion. Nucleotides -220 to -120 relative to the lectin gene transcription start site⁵ are shown to the right of the brackets. Boxes: binding sites, determined by their positions relative to a known DNA sequence ladder.

from DNase I digestion in the presence of embryo nuclear proteins (compare lanes F and B). Region I is 12 base pairs (bp) long, represents nucleotides -184 to -173 relative to the 5' lectin gene end (box I, Fig. 4*d*), and was protected fully from DNase I digestion. By contrast, region II is 40 bp long, represents nucleotides -165 to -126 relative to the lectin gene transcription start site (box II, Fig. 4*d*), and contains two DNase I protection domains. One domain, designated IIA, was protected strongly from DNase I digestion with embryo nuclear proteins. On the

other hand, sequences flanking the IIa domain were protected less completely (lane B, Fig. 4d).

Sequence comparison of regions I and II indicated that they share a seven-nucleotide core motif 5' ATT^AAAT 3'. This motif constitutes most of the region I nucleotides and is localized in the strongly protected region IIa domain. Neither pBR322 nor the leghaemoglobin 5' gene region contain the core sequence. By contrast, the core motif is present in the KTi1 and KTi2 Kunitz trypsin inhibitor 5' gene regions as predicted from the protein gel blot binding studies of Fig. 3. We showed recently that embryo nuclear protein protects the Kunitz trypsin inhibitor core motif from DNase I digestion, and that lectin and Kunitz trypsin inhibitor 5' regions compete for the same DNA binding protein (C. Reeves and R. B. G., unpublished results). Presumably this is the 60K nuclear protein identified in the experiments of Fig. 3. Together, these findings indicate that DNA sequences within a 60-bp lectin gene 5' region interact with an embryo DNA binding protein, and that these sequences mediate the formation of an analogous protein-DNA complex with a second seed protein gene that has a similar developmental-specific expression program.

We have shown that soybean embryos contain a 60K DNA binding protein that interacts with the lectin and Kunitz trypsin inhibitor regions (Fig. 3). We have no direct evidence that the embryo DNA binding protein or the sequence that it interacts with are important for regulating seed protein gene transcriptional activity. Recent gene transfer studies indicate, however, that only 0.5 kb of 5' sequence is necessary to program lectin gene expression during tobacco seed development (J.K.O. and R.B.G., unpublished results). Similarly, analogous studies showed that only 0.4 kb 5' to the KTi2 Kunitz trypsin inhibitor gene is required for expression in developing seeds (K.D.J. and R.B.G., unpublished results). We cannot exclude the possibility that developing embryos contain lower prevalence, seed protein gene-specific DNA binding proteins that were undetected by our procedures. Nor can we rule out that there are other gene regions that are important for regulating seed protein gene expression during embryogenesis and in mature plant organ systems. However, our observations that the embryo DNA binding protein appears and disappears in parallel with fluctuations in seed protein gene transcriptional activity, and that the DNA binding protein interacts with similar sequences flanking two distinct seed protein genes, suggest that the 60K embryo nuclear protein is involved in regulating seed protein gene expression. Clearly, the exact nature of the embryo DNA binding protein and its function in developmental-specific seed protein gene expression remain to be determined.

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Active sliding between cytoplasmic microtubules

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Microtubules are versatile cellular polymers that play a role in cell shape determination and mediate various motile processes such as ciliary and flagellar bending, chromosome movements and organelle transport¹. That a sliding microtubule mechanism can generate force has been demonstrated in highly ordered structures such as axonemes^{2,3}, and microtubule-based force generation almost certainly contributes to the function of mitotic and meiotic spindles^{4,5}. Most cytoplasmic microtubule arrays, however, do not exhibit the structural regularity of axonemes and some spindles, and often appear disorganized. Yet many cellular activities (such as shape changes during morphogenesis⁶⁻⁹, axonal extension^{10,11} and spindle assembly) involve highly coordinated microtubule behaviour and possibly require force generated by an intermicrotubule sliding mechanism, or perhaps use sliding to move microtubules rapidly into a protrusion for stabilization¹². Here we show that active sliding between cytoplasmic microtubules can occur in microtubule bundles of the amoeba *Reticulomyxa*. A force-producing mechanism of this sort could be used by this organism to facilitate the extension of cell processes and to generate the dynamic movements of the cytoplasmic network.

The giant freshwater amoeba *Reticulomyxa* contains a dynamic peripheral network of interconnected fine cytoplasmic strands, each supported by a bundle of parallel microtubules and microfilaments^{13,14}. Strands extend from the cell body at rates often exceeding 5 $\mu\text{m s}^{-1}$ and readily branch from, fuse with or laterally translocate along each other^{13,14}. In extensive flattened areas of network, the microtubule bundles themselves show an analogous dynamic behaviour, similar to the reticulopodial motility described for *Allogromia*^{15,16}. We have found¹⁷ that reactivated lysed cell models of *Reticulomyxa* also exhibit an active bending and splaying of microtubule bundles, suggesting that the motive force for this motility is generated by intermicrotubule interactions. The degree of splaying ranges from a moderate 'looping-out' of microtubules from large bundles (Fig. 1) to the formation of a dense, random microtubule meshwork (see Fig. 8 of ref. 17).

To examine further this striking behaviour of microtubules, we devised an experimental model in which microtubule sliding,

Table 1 Inhibitor effects on reactivated sliding

Treatment		Microtubule sliding
Vanadate	100 μM	Complete inhibition
	50 μM	Nearly complete inhibition
	10 μM	No effect
EHNA	5 mM	No effect
	2.5 mM	Complete inhibition
NEM	0.1 mM	Slight rate inhibition
	50 $\mu\text{g ml}^{-1}$	Complete inhibition
Trypsin	10 $\mu\text{g ml}^{-1}$	No effect

Sodium orthovanadate and EHNA were added to lysed networks with the ATP. The sulphydryl reagent NEM was added before the ATP, incubated for 5 min, and followed with a 5-min incubation of 5 mM dithiothreitol. Trypsin was incubated for 3 min at either concentration, followed by extensive washing and reactivation. No visible changes in network morphology at the light-microscope level result from any of these inhibitor treatments. Results were obtained from at least three preparations (total of at least 10 different ends) at each concentration.

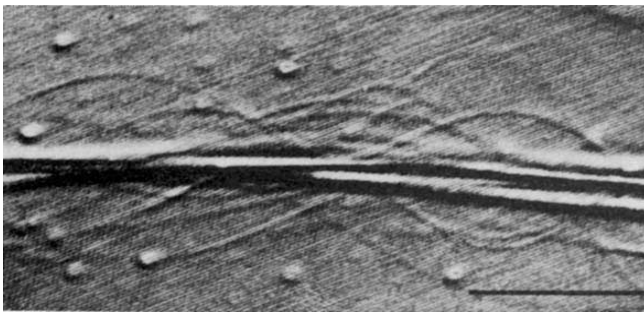


Fig. 1 Video-enhanced differential interference contrast micrograph of a lysed strand 4 min after reactivation. Note how the microtubules appear to loop away from the large bundle. In many areas, the loops become large and entangle other strands, forming a microtubule meshwork. Scale bar, 10 μm .

if it occurs, would be translated into telescoping. We cut lysed networks into a series of 20–50- μm long segments with a glass micropipette, stripped them of organelles with 1% Triton X-100, and reactivated them with ATP. Immediately after adding ATP, microtubules extruded rapidly from the ends of cut bundles (Figs 2, 3). Both during and after this extension, the bundles themselves laterally dissociated into 'free' microtubules.

We observed microtubule extension at the proximal and distal edges of the cut, and it is often simultaneous. Of 95 bundle ends, 84 actively protruded microtubules. In bundles estimated to contain 20–25 microtubules, between 3 and 15 microtubules would extend. Many microtubules were completely ejected from the bundles and either floated in solution or adhered to the

coverslip. Neither the ejected microtubules nor the bundles themselves ever glided or crawled across the surface, as has been reported for other cell systems^{18,19}. Rates of extension ranged from 0.5 to 10 $\mu\text{m s}^{-1}$, and the length increased from 0.1 to 3 $\times$ the original length of the cut bundle. These large variations in the number of microtubules extending from the bundles, and the rate and extent of telescoping, make quantitation difficult: there seems to be no correlation between bundle length or microtubule number and the degree of sliding.

Our inhibitor studies (Table 1) demonstrate that the microtubule telescoping and reactivated organelle movement have similar sensitivities to vanadate, Erythro-9-[3-(2-hydroxynonyl)] adenine (EHNA) and *N*-ethylmaleimide (NEM)¹⁷. Therefore, we cannot say with certainty whether organelle movements and microtubule sliding are brought about by the same motor, or by two different motors with similar pharmacological properties. Disruption of the actin-filament network of the cell with gelsolin and 20 μM free calcium does not inhibit reactivated sliding, so microfilaments seem not to play a significant role in this phenomenon.

As well as sliding, microtubules detach from the sides of the bundle and float free in solution, leading to a complete dissociation of the remaining (non-sliding) microtubules in smaller bundles, and the cessation of further sliding. It is likely that these two phenomena are different manifestations of the same process. Whether a microtubule slides or dissociates perhaps depends on the number and arrangement of active crossbridges. Similar ATP-sensitive bundling and dissociation phenomena of microtubules have recently been described²⁰.

Ultrastructural studies of microtubule arrangements in native bundles and lysed cell models reveal an average minimal dist-

Fig. 2 *a-c, d-f, g-i*, Three different video sequences of microtubule sliding. *a, d, g*, before ATP addition; *b, c, e, f, h, i*, after addition. Numbers, time (s) after ATP addition; arrows, original end positions. In these preparations, microtubules hover just out of the plane of focus either beyond the original ends or along the sides of the bundle after ATP is added. Because the focal plane of DIC optics is so shallow, it is difficult to document all the microtubules that have telescoped out of the bundle in a single photograph. They can be very easily seen by focusing through the preparation.

Methods. Amoeba were grown on coverslips and lysed with half-strength PHEM buffer (30 mM PIPES, 12.5 mM HEPES, 4 mM EGTA and 2 mM MgCl_2) containing 0.15% Brij 58, 5% hexylene glycol, and 1 mM vanadate as described in ref. 17. Lysed strands were cut on a Zeiss IM 35 inverted microscope with a micropipette pulled out to a tip diameter of 1–2 μm and mounted in a Leitz micromanipulator. The coverslips were inverted over spacers fixed to a slide, forming perfusion chambers, and the cut areas were relocated in a Zeiss Photomicroscope III. Cut bundles were extremely stable, with no changes in length or diameter observed for at least 30 min (longest time examined). The cut strands preserved their native orientation, thus allowing a distinction between proximal and distal ends. Nearly all the native organelles could be removed from the bundles with a brief wash of 50% PHEM containing 1% Triton X-100. To control against the possibility that the few remaining 'active' organelles pushed microtubules out of the bundles, a few preparations were briefly rinsed with 300 mM KCl before reactivation. This treatment removed all organelle motility but had very little effect on microtubule sliding. Microtubule sliding was initiated by the addition of 1 mM ATP in 50% PHEM and was most prominent within the first 30 s of reactivation. Microtubule number was estimated by counting visible microtubules on the video screen after bundle dissociation and from previous correlative light and electron microscopy of intact strands²⁸. The video-processing steps are as described in ref. 17. Scale bar, 10 μm .

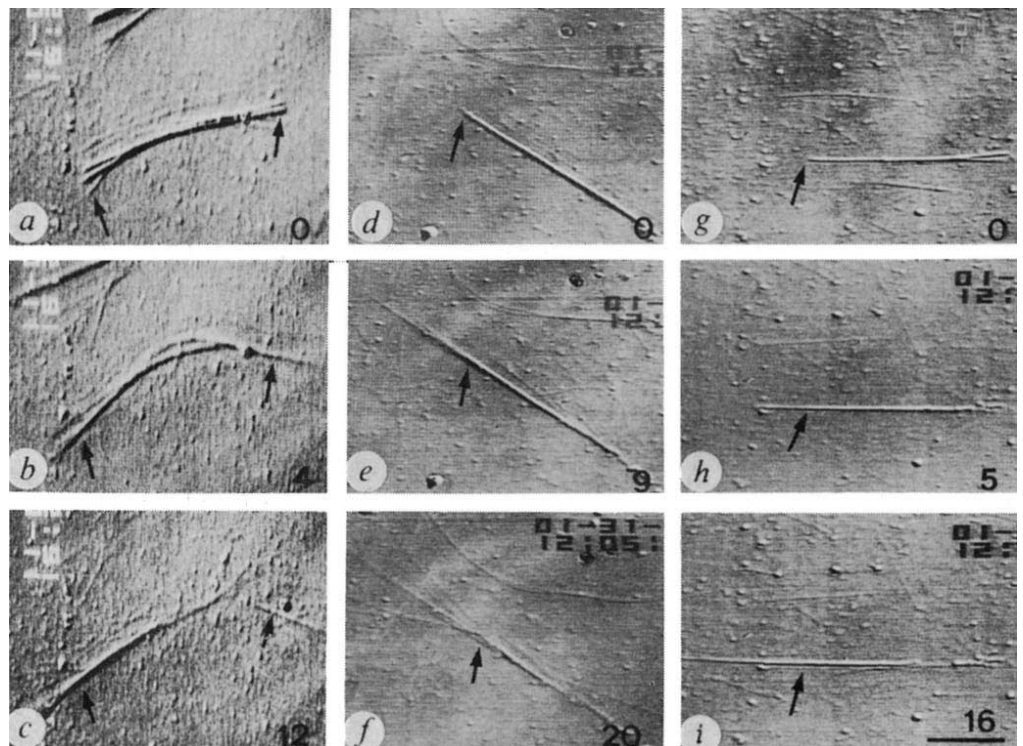
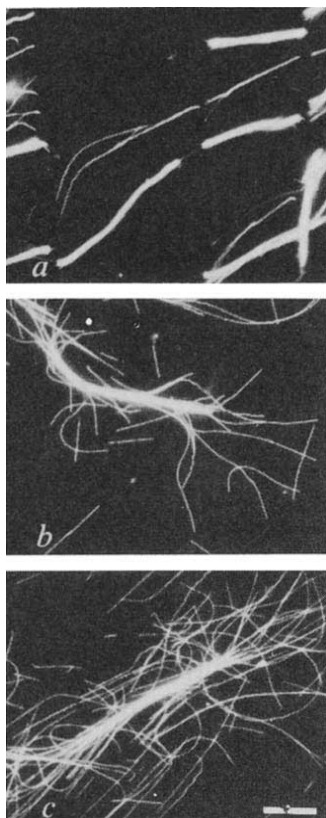


Fig. 3 Lysed and cut strands seen by indirect immunofluorescence microscopy with a monoclonal antibody directed against *Reticulomyxa* α -tubulin. (see ref. 13 for staining methods). *a*, Several bundles fixed after cutting, without ATP addition. These bundles are comparable in length and diameter to the segments shown in Fig. 2. *b*, A slightly thicker bundle than *a* fixed after a 10-s exposure to ATP. Several microtubules now emanate from the original segment. The curvature of the microtubules is a consequence of flushing through the fixative. Microtubules with at least one free end always appear relatively straight in live preparations. Note that many more microtubules may have been present in the unfixed preparation, as most 'released' microtubules would have been rinsed away. *c*, Another bundle fixed after a 10-s exposure to ATP, demonstrating a more advanced state of sliding. Scale bar, 10 μ m.



ance of 20 nm between microtubules (see, for example, Fig. 4 of ref. 13). Note that a similar spacing exists in various other motile microtubule arrangements²¹. In negatively stained lysed strands, many globular projections from, and connections between, microtubules can be observed (data not shown). Brief trypsin treatments, sufficient to block sliding (Table 1), reduce (but do not remove completely) the projections, suggesting that they may represent 'crossbridges' responsible for force generation. More detailed experiments on the nature, function, molecular structure and distribution of these putative crossbridges are necessary to investigate this possibility.

Using the simple trick of pre-cutting the long microtubule bundles of *Reticulomyxa* networks into short segments, we have shown here directly that microtubules can slide past each other in a manner reminiscent of trypsin-treated flagellar axonemes². This form of inter-microtubule motility readily explains the dramatic ATP-dependent splaying and bending of microtubule bundles observed *in vitro*¹⁷, and probably forms the molecular basis of network dynamics and extension *in vivo*^{13,14}, which cannot be explained by conventional microtubule polymerization kinetics. The molecular nature of the motor responsible for microtubule sliding is presently unknown. *Reticulomyxa* is not known to express ciliated or flagellated forms, so microtubule sliding is unlikely to be caused by a precursor of dynein, the force-generating protein of axonemes. The inhibitor profile (Table 1) also provides evidence against this possibility. The sensitivities to different inhibitors are, however, similar to those of a high-molecular-mass ATPase found in *Reticulomyxa* (U.E. *et al.*, in preparation), which is distinct from dynein or kinesin, another force-generating protein of non-muscle cells²².

Inter-microtubule sliding has been demonstrated to occur during ciliary and flagellar movement²; in the elongation of isolated diatom spindles²³; in protozoan axostyle motility^{24,25}; and in some ciliates²⁶, processes all based on rigid, 'paracrystalline' microtubule arrangements. *Reticulomyxa* microtubule bundles, on the other hand, are comparatively loosely organized and highly dynamic. A tightly ordered array of microtubules is therefore not a prerequisite for their ability to interact and move.

An analogy can perhaps be drawn with actomyosin systems. In striated muscle, actin and myosin interdigitate in a highly ordered fashion, while non-muscle cells contain relatively isotropic arrays. Both arrangements, however, are able to generate tension and contract.

There seems to be a fundamental difference in sliding mechanisms between axonemal-type structures and mitotic spindles. Microtubules are aligned with a uniform structural polarity in axonemes, whereas sliding in spindles is believed to occur in areas where microtubules of opposite polarity interdigitate²⁷. Preliminary investigation of microtubule polarity in *Reticulomyxa* indicates that the vast majority of microtubules share the conventional 'plus-end-distal' polarity (data not shown). In this respect, the sliding observed here probably results from the interaction between microtubules of the same polarity.

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Crystal structure of the intensely sweet protein monellin

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Two unusual proteins, discovered in African berries, possess the interesting property of having a very high specificity for the sweet receptors. These proteins, monellin^{1,2} and thaumatin³, are approximately 100,000 times sweeter than sugar on a molar basis and several thousand times sweeter on a weight basis. Neither contains carbohydrates or modified amino acids. Several interesting observations have been made about the two proteins: native conformations are important for the sweet taste⁴⁻⁶, although both proteins are intensely sweet, there are no statistically significant sequence similarities between them⁷; and despite the absence of sequence similarity, antibodies against thaumatin compete for

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monellin (as well as many other sweet compounds^{8,9}, but not for chemically modified non-sweet monellin⁹) and vice versa¹⁰. To understand the structural basis of these observations we determined the crystal structure of thaumatin¹¹, and report here the structure of monellin at 3 Å resolution. Monellin consists of two peptide chains, the A chain of 44 residues and the B chain of 50 residues^{12,13}. We find no similarity between the backbone structure of monellin and that of thaumatin.

Of the two crystal forms^{14,15} of monellin, the more stable monoclinic form is in space group $P2_1$. The unit cell ($a = 39.6$ Å, $b = 71.8$ Å, $c = 86.9$ Å and $\beta = 107.1^\circ$) contained four monellin molecules, a total of eight peptide chains per asymmetric unit. A 3.0-Å data set for the native crystal and 3.5-Å data sets for five heavy atom derivatives have been obtained using an ω -step scan on a Nicolet diffractometer at 4 °C. The heavy atom soaking conditions and refinement statistics are shown in Table 1. A multiple isomorphous replacement (MIR)¹⁶ electron density map showed molecular boundaries similar to those found in the 5.5-Å map¹⁷, as well as some recognizable β -sheets, but it was not possible to trace the peptide backbone unambiguously even after noise filtering¹⁸ and phase extension to 3.0-Å resolution. An examination of the preliminary tracing of the four independent monellin molecules revealed three local symmetry axes: one pseudo 2-fold screw axis (a rotation of 156.1° and a translation of 29.0 Å) relating two pairs of molecules and two local 2-fold axes (a rotation of 180°), one in each pair of molecules. Finally, starting with the 3.5-Å MIR map, electron density averaging¹⁹ around the two local 2-fold axes only, without the screw averaging, and then phase extension to 3.0 Å, resulted in an improved electron density map (Fig. 1; Table 2). This map allowed an unambiguous tracing of the backbone structure. Two amino-terminal residues of the A chain and three carboxyl terminal residues of the B chain are disordered and could not be seen in the map.

The backbone foldings of the two pairs of molecules (not averaged by local screw axis) in an asymmetric unit are very similar (Fig. 2). There is one five-stranded, anti-parallel β -sheet made of two strands from the B chain (one continuous, B1–5; the other of two segments B35–38 and B41–47, separated by a three-residue bulge), and the remaining three from the A chain (A5–15, A18–27, A30–39). The β -sheet is twisted in the same handed sense as observed in β -sheets of many other proteins^{20,21}.

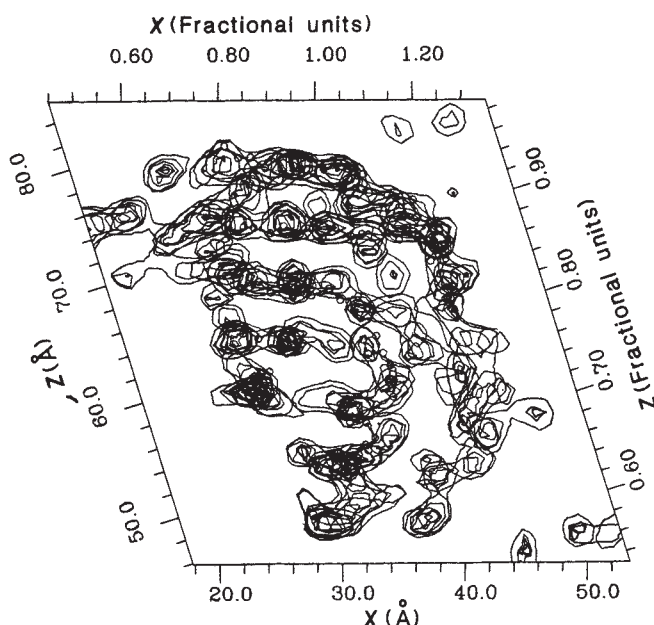


Fig. 1 Thick (9 Å) section of electron density map (2-fold averaged and phase extended) showing the molecular boundary, most of the β -sheet (four connected electron density contours parallel to the X-axis), and α -helix (lower middle).

The only α -helix (B10–25) is located in the gently twisted concave side of the β -sheet, and runs almost perpendicular to the β -strands.

This 3.0-Å structure provides explanations for several previous biochemical observations. First, the two chains can be separated only by very strong denaturation^{4,13}. Second, the chemical reactivity of the cysteine (B41) is very low but that of the neighbouring methionine (B42)^{5,6} is high. Third, fraction of monellin molecules can be cross-linked into dimers in solution²². This last is probably because the top two strands of the β -sheet of one molecule and the corresponding strands on the local 2-fold-related molecule are near each other and have extensive hydrophobic contact (Fig. 2), and the 'back' sides of the two

Table 1 Heavy-atom derivatives

	Concentration (mM)	Soak time (days)	No. of sites	Positions (x, y, z)	Occupancies (e)	Temp. factors ($\text{\AA}^2$)	R-Cullis*	Phasing power†
KAuCl ₄	0.33	4	4	0.0116, 0.0064, 0.1095	29.5	50	0.65	1.54
				-0.0090, 0.4646, 0.3649	23.1	46		
				0.0949, 0.0108, 0.1225	9.6	26		
				0.5129, 0.0844, 0.3645	8.3	47		
Pt((NH ₂) ₂ (CH ₂) ₂)Cl ₂	0.33	18	2	-0.0121, 0.4464, 0.3566	45.3	56	0.51	2.21
				0.0092, 0.3567, 0.3372	8.4	48		
HgCl ₂	0.33	3	2	0.9177, 0.0531, 0.4796	23.0	67	0.68	0.81
				0.8770, 0.0674, 0.5012	8.4	48		
C ₂ H ₅ HgCl	0.70	11	6	0.9408, 0.0498, 0.7125	20.9	47	0.56	2.26
				0.8185, 0.0350, 0.6722	18.4	47		
				0.7779, 0.4544, 0.4174	30.6	55		
				0.0341, 0.1454, 0.1638	14.3	48		
				0.9041, 0.1169, 0.1592	16.0	42		
				0.0997, -0.0498, 0.5304	11.8	59		
K ₂ PtCl ₄	2.0	5	3	0.9842, 0.4610, 0.3596	17.0	46	0.67	0.77
				0.3417, 0.4827, 0.4861	16.8	63		
				0.3941, 0.0556, 0.1118	11.6	63		

* $R\text{-Cullis} = \Sigma |\Delta F - F_H| / \Sigma \Delta F$ for centric reflections; $\Delta F = |F_{PH} - F_P|$.

† Phasing power is the root-mean-square calculated heavy atom structure factor divided by the root-mean-square lack of closure error (r.m.s. F_H /residual).

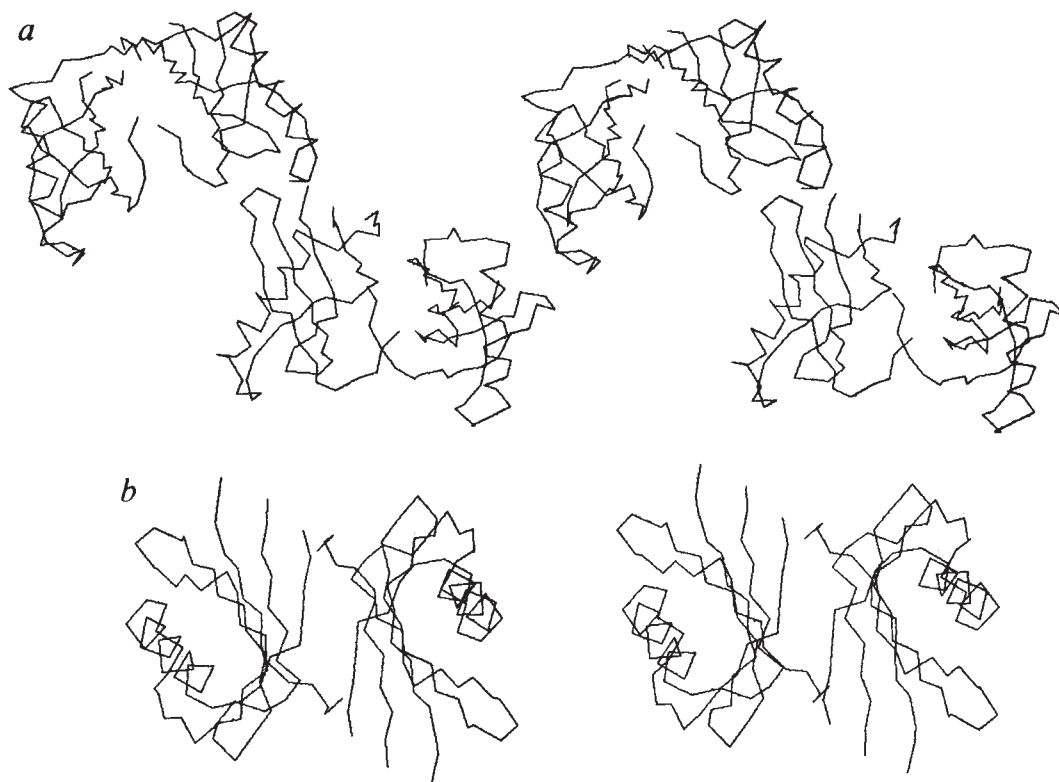


Fig. 2 *a*, Stereo drawing of four monellin backbone structures in an asymmetric unit of a unit cell. The pseudo 2-fold screw axis is running vertically. *b*, Stereo drawing of one pair of the backbone structures of monellin molecules. The viewing direction is along the local noncrystallographic 2-fold symmetry axis.

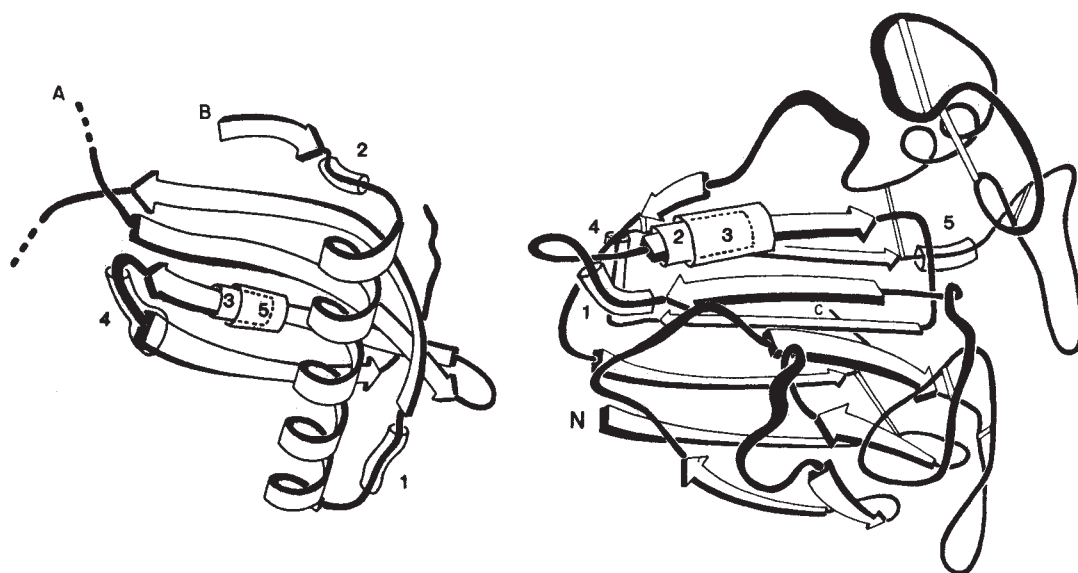


Fig. 3 Schematic drawings of the backbone structures of monellin (left) and thaumatin (right). Tubed sections, regions with tripeptide sequence similarities between the two proteins. The homologous tripeptides of each pair are identified by the same number in both structures. Broken lines, disordered residues that could not be seen in the electron density maps.

β -sheets almost form a β -barrel.

Despite the fact that monellin and thaumatin share unusual properties of being sweet and immunologically cross-reactive, there is no similarity of significance in amino-acid sequence and three-dimensional backbone structures. Thaumatin (Fig. 3), 207 residues in a single chain, is made of a core of two β -sheets, one on top of the other, and two large complex looped 'domains'¹¹. Each β -strand is only six amino acids long on average. Monellin, on the other hand, has a single β -sheet of five strands, each strand containing an average of ten residues. Monellin has a few small tight loop regions as compared to several large loops with disulphide bonds in the thaumatin molecule. Thus in overall structure there appears to be little similarity between the two molecules.

At the level of small local conformation, we examined five pairs of homologous tripeptide sequences: residues B28-30, B6-8, A22-24, A29-31 and A21-23 in Monellin (labelled 1 to

5 in Fig. 3) have homologous sequence at residues 94-96, 100-102, 101-103, 118-120 and 128-130 (also labelled 1 to 5) in thaumatin, respectively⁷. Although the tripeptide homologies are not statistically significant, the immunological cross reactivity implies the existence of common chemical and structural features, and the possibility exists that a joint site made of several of these short homologous regions may act as an antigenic determinant. Conformations of three of the five pairs, regions 1, 4 and 3 of monellin, are found to be similar to their counterparts in thaumatin at the current resolution of 3 Å: the first two are located in looped regions and the third in a β -strand. Of these, region 3 was not considered to be an antigenic site because it is within the β -sheet, and only the side chain of A23 points toward the exterior of the protein.

Three conclusions can be drawn. First, despite the fact that both proteins are intensely sweet, there is no similarity of apparent importance in the overall backbone structures of the

Table 2 Solvent flattening and phase extension

Cycle	1*	2†	3‡	4†	5‡	5a§
Resolution (Å)	3.5	3.5	2.5	3.5	3.5	3.5–3.0
Reflection no.	5411	5411	5411	5411	5411	2588
Figure of merit ¹⁶	0.68	0.72	0.87	0.91	0.93	0.61
R-factor	—	—	0.24	—	0.22	0.39
$\Delta\alpha^\circ$	—	33.2	39.8	47.3	48.0	—
Correlation coefficient between:						
molecules 1 and 2	0.51	—	0.84	—	0.87	—
molecules 3 and 4	0.46	—	0.85	—	0.87	—

$R^2 = \Sigma(F_{\text{obs}} - F_{\text{calc}})^2 / \Sigma F_{\text{obs}}^2$, $\Delta\alpha^\circ$ = Average accumulated phase difference.

* Original MIR data.

† After electron density averaging around two local 2-fold axes.

‡ After 2-fold averaging and solvent flattening.

§ After solvent flattening and phase extension, data from 3.5 to 3.0 Å resolution.

|| $\Sigma(\rho_a - \bar{\rho}_a)(\rho_b - \bar{\rho}_b) / (\Sigma(\rho_a - \bar{\rho}_a)^2 \times \Sigma(\rho_b - \bar{\rho}_b)^2)^{1/2}$, where ρ_a is electron density for molecule a ; ρ_b , electron density for the symmetry related molecule b ; $\bar{\rho}_a$, $\bar{\rho}_b$, average electron densities of the respective molecules.

proteins. Second, at the local level, monellin and thaumatin have only two small regions (both tripeptides), located in exposed, looping regions, sharing sequence and topology. According to the conventional notion, each of these regions is too small by itself to be the complete antigenic determinant. Third, because the separations of these two regions are different in the two proteins (26 Å in monellin and 18 Å in thaumatin), we can rule out the possibility that they jointly form the common single antigenic site. The only remaining possibility is that several smaller regions (shorter than three residues), with or without one of the two above tripeptides, jointly form the common antigenic site.

As for sweet taste, there is no *in vitro* assay and no taste receptor molecule has yet been isolated. However, the *in vitro* immunological cross-reactivity may cast some light on taste. The possible relevance is suggested by the facts that (1) the antibodies of one protein cross-react to the other and vice versa^{8–10}; (2) antibody–protein complexes lose their ability to elicit sweet taste (unpublished results); (3) a group of sweet compounds such as aspartame compete for the same antibodies but non-sweet aspartame derivatives do not^{8,9}; (4) cross-adaptation of monellin and thaumatin in human taste experiments²⁵ and electrophysiological experiments²⁴ suggests they are recognized by the same receptor; (5) because antibody and receptor binding sites are likely to be exposed, one may be a subset of the other.

At present these are the only two proteins with known crystal structures that have immunological cross-reactivity without sequence similarity. Biochemical and immunological studies are in progress to identify the regions which may be responsible for the antibody cross-reactivity and the sweet-taste-receptor binding. High-resolution refinement of both proteins is also in progress. The α -carbon coordinates of the structure will be deposited in the Brookhaven Protein Data Bank.

We thank A. de Vos, S. Holbrook, G. Smith and E. Harvey (University of California, Berkeley), for help and advice with computer hardware and software; B. C. Wang (University of Pittsburgh) for his ISIR program; and J. L. Smith and W. Hendrickson (Columbia University) for programs and advice on averaging electron density maps. This research has been supported by grants from the NIH and by the NutraSweet Co., Mount Prospect, Illinois.

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Corrigendum

Cloning and sequencing of a cDNA for a ligninase from *Phanerochaete chrysosporium*

Ming Tien & Chen-Pei D. Tu
Nature **326**, 520–523 (1987)

FURTHER work on sequencing of ligninase cDNA clone ML-1 has led to the following corrections in the nucleotide sequence:

a change from CG to GC at positions 125–126;
a change from G to C at position 1,043;
an insertion of a C at position 1,051.

The above changes in the nucleotide sequence result in the following correction in the amino acid sequence:

a change from Ser 14 to Cys 14;
a change from Ser 320 to Thr 320;
a change of amino acids 323–346 to (323)Pro-Thr-Leu-Thr-Thr-Leu-Pro-Gly-Pro-Glu-Thr-Ser-Val-Gln-Arg-Ile-Pro-Pro-Pro-Pro-Gly-Ala-STOP(344).

The new termination codon is TAA at positions 1116–1118. The change in reading frame at nucleotide residue 1051 decreases the total amino acid residues from 346 to 344.

Errata

The surface windfield over the Antarctic ice sheets

Thomas R Parish & David H. Bromwich
Nature **328**, 51–54 (1987).

FIGURES 2 and 3 in this letter were transposed. The upper figure on page 53 is Fig. 3, including arrows to show wind direction.

Electrogenic glutamate uptake is a major current carrier in the membrane of axolotl retinal glial cells

Helen Brew & David Attwell
Nature **327**, 707–709 (1987).

IN the penultimate paragraph of this letter (beginning “Depolarization induced release . . .”) two words were omitted during the proof correction stage. The last sentence in the paragraph should read: “This release, which may be non-vesicular, could be accounted for by the strong voltage-dependence of glutamate transport which we have observed, if the same carrier exists in neurons and if the glutamate concentration in neurons is high enough to allow a net glutamate efflux on depolarization.”

Doppler colour flow imaging

C.R.B. Merritt

Visualization of the vascular system, and the assessment of blood flow and organ perfusion are several applications of this emerging area of medical imaging.

DIAGNOSTIC ultrasonography is based on the production of images by the reflection of high frequency sound (usually in the range of 5–10 MHz) from tissue interfaces in the body. For conventional ultrasound imaging, pulse-echo transmission, detection, and display techniques are used. The amplitude information in the returning signal is used to generate an image in which echo amplitude is displayed in grey scale. The echoes forming the image come from stationary targets within the body because rapidly moving targets, such as red cells flowing in the blood stream, produce echoes of low amplitude that are usually not detected. When sound impinges on a stationary reflector, the backscattered ultrasound has essentially the same frequency as the transmitted sound. If, however, the reflecting interface is moving with respect to the source of the sound, there is a change in the frequency of the sound scattered from the target. This change in frequency is directly proportional to the velocity of the reflecting interface, and is explained by the Doppler effect (Fig. 1). The effect is identical to the audible change in pitch of a train whistle, that increases in frequency as the train approaches, and decreases in frequency as the train moves away from the listener. Just as the change in pitch of the train's whistle can be used to infer the speed of the train, so can the change in frequency of ultrasound indicate the relative velocity of red cells flowing within a vessel. With diagnostic ultrasound instrumentation, Doppler frequency data may be displayed as an audible signal for analysis by ear, or in graphic form as a time-varying plot of the frequency spectrum of the returning signal, using a fast Fourier transform to perform the frequency analysis. If the angle of the vessel axis to the ultrasound beam is known, accurate calculation of flow velocity is possible.

Until recently, the ultrasound instruments used most often for vascular evaluation have been duplex scanners. These combine real-time imaging of stationary interfaces, that outline the vessel wall and surrounding tissues, with a graphic display of Doppler frequency spectra obtained from small sample volumes of flow within the vessel lumen¹. Although highly useful, these systems are limited because the flow information is obtained only from the highly restricted region from which the Doppler sample is obtained, and is not evaluated in the remainder of the image. Thus, to obtain maximum information

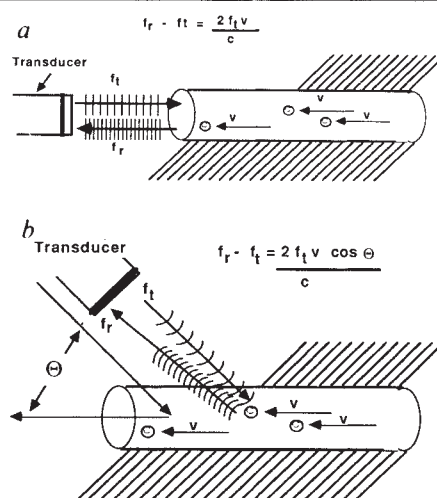


Fig. 1 If the ultrasound is transmitted along the axis of the vessel (a) then the velocity of the red cells, v , can be related to the shift in Doppler frequency $f_r - f_t$, where f_r is the frequency of the reflected ultrasound, f_t is the frequency of the transmitted ultrasound, and c is the velocity of sound in the medium. If the ultrasound beam approaches the vessel at an angle θ (b), then the frequency of the reflected ultrasound f_r is reduced in proportion to the cosine of θ .

with duplex Doppler ultrasound, a skilled operator must perform a careful and time-consuming sampling of sites within the vessel lumen where flow disturbances are likely to be found². A more ideal method that allows display of flow characteristics throughout the entire image, and provides simultaneous high resolution imaging of the vessel wall and adjacent tissue features is now available — Doppler colour flow imaging, or angiodynography.

Going with the flow

The first instruments to allow real-time display of flow data in the image, rather than a spectral display, were developed in Japan for evaluation of cardiac blood flow, and initial clinical results were reported in 1982³⁻⁵. These instruments placed emphasis on the display of flow data rather than the simultaneous display of tissue and flow information, which is of particular importance in noncardiac applications. The first instrument designed specifically for simultaneous tissue and flow imaging in peripheral vascular, abdominal, and pelvic applications was placed into clinical evaluation in 1985, and has become commercially available within the last year (Quantum AngioDynograph, Quantum Medical Systems, Issaquah, Washington). This system uses a linear

phased array to detect echo amplitude, phase and frequency, and processes this information in real time to generate the image. Stationary or slowly moving targets provide the basis for the grey-scale B-mode image. Signal phase provides information about the presence and direction of motion, and changes in echo signal frequency indicate the velocity of the reflecting target. All of these data are processed by a high-speed dedicated array processor that updates the image display at up to 18 times per second, for real-time assessment. Continuous focusing is provided by the phased array to give high resolution grey-scale images of tissues as well as very small Doppler sample volumes (0.6×1.5 mm at 7.5 MHz and 0.9×2.0 mm at 5.0 MHz) throughout the image. Backscattered signals from red blood cells are displayed in colour as a function of their motion toward or away from the transducer. The degree of the saturation of colour is used to indicate the relative velocity of the moving red cells, with less colour saturation indicating higher velocity. In addition to the detection of flow data from each pixel in the image, the system also has dual range-gated pulsed Doppler with spectral analysis for display of conventional Doppler data.

Simultaneous display

With Doppler colour flow imaging, it is now possible to provide the simultaneous real-time display of a high resolution 2-dimensional grey-scale image and Doppler flow. This capability has immense clinical potential in abdominal, pelvic, obstetrical and oncological applications, and in evaluation of the carotid and peripheral vascular systems. Although clinical experience is limited due to the short time these systems have been available, the significant advantages of Doppler colour flow imaging compared to duplex Doppler instrumentation are emerging. These include global Doppler sampling, reduced aliasing artefact, ease of measurement of Doppler angle to allow more accurate estimation of velocity, simultaneous real-time Doppler and tissue information, and ease of operation.

Our initial experience using Doppler colour flow imaging in a number of clinical applications has given us a highly positive impression of this new method of ultrasound imaging⁶. The ability to image simultaneously vascular flow and tissue permits simple, rapid and accurate evaluation of large vessels for stenosis, occlusion

and flow disturbance. Most significant abnormalities can be detected from the image alone, but quantitative measurement of flow characteristics is also possible with this system. The technique also shows promise for the evaluation of the vascular supply of abdominal and pelvic organs, and for obtaining clinically useful information related to organ perfusion and tumour neovascularity. With this technique, we may finally begin to see some useful degree of ultrasound tissue characterization based on tissue perfusion patterns. □

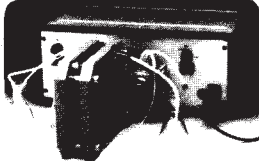
Christopher R. B. Merritt is at the Department of Diagnostic Radiology, Ochsner Clinic, 1514 Jefferson Highway, New Orleans, Louisiana 70121, USA. For more information, fill in reader service number 100.

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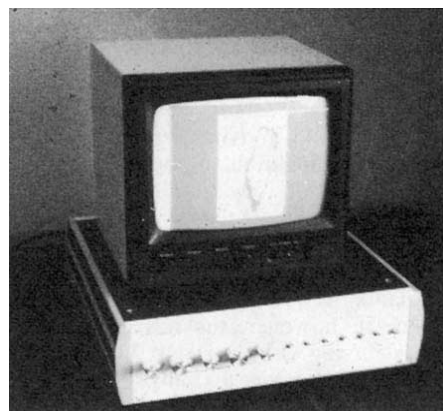
An intracellular ion measurement system, a cell length monitor, and a new perspective on NMR are part of this week's feature on the discipline where physics meets biology.

CALCIUM ions have attracted interest of late by virtue of their action as intracellular second messengers. Hamamatsu Photonics Europa has developed a 2-dimensional **intracellular calcium ion measurement system** to enable researchers to collect data from fluorochrome stained samples (*Reader Service No. 101*). The system consists of a video camera equipped with a silicon intensified target, an image processor with a $688 \times 512 \times 16$ bit memory, and an IBM PC/AT plus associated software. Hamamatsu says the system's camera can detect light levels down to 10^{-4} lux with low background noise. The image processor is capable of performing image manipulations such as averaging, subtraction, zooming, histograms and intensity profiles, and it has a scaler, timer, 256-pseudo-colour display and remote control.

Sterilin distributes a line of Nucleopore polymer bonded membranes and holders for **chemotaxis studies** (*Reader Service No. 102*). The membranes have cylindrical pores, manufactured by fission element bombardment, that Sterilin says facilitate rapid cell migration and reduce incubation time. The membranes are thin, to allow slower moving cells, such as monocytes and macrophages, to be studied. The membranes are available with or without the wetting agent PVP, and come in sizes from 13 mm to 293 mm in diameter, with pore sizes from $0.01 \mu\text{m}$ to $12 \mu\text{m}$. Prices range from £35 to £80 (UK) for packs of 100.

Varian has teamed up with Sun Microsystems to offer a new line of **NMR data-systems** for Varian's VXR series of research NMR spectrometers and VIS family of *in vivo* imaging NMR spectroscopy systems (*Reader Service No. 103*). The new VXR 5000 series is based on the Sun-3 family of workstation computers, and come with Varian's NMR software over a UNIX operating system shell. All tasks relating to spectrometer control, such as pulse sequence control, automatic magnet shimming, or sample management, benefit from the series' computing speed of 1.5 to 4 million instructions per second (MIPS). The software has a 2-dimensional analysis tool that automatically finds and annotates cross peaks in 2-dimensional correlated spectra and develops a connectivity table, and that has an interactive deconvolution capability to unravel overlapping peaks. The VXR 5000 series is priced from \$39,500 (US).

HVS Image Analysing Ltd has a **cell-length monitor** especially suited for measuring the contractions of heart muscle cells perfused with stimulants (*Reader Service No. 104*). The SP144 performs non-contact measurements of a cell 50 times per second, enabling it to follow contractions in real time. The system



The HVS Image Analysing monitor specializes in the contraction of heart muscle.

includes a monitor and an image analysis unit, and is meant to be used with a microscope equipped with a video camera. Outputs for length and rate of change of length are provided for connection to a chart recorder. Interfaces to microcomputers are an optional extra. The SP144 carries a \$5,750 (US) price tag.

A multiple excitation **spectrofluorometer system**, the Series 300, has been developed by H & L Instruments for intracellular measurements using the biological probes furan-2, indo-1 and BCECF (*Reader Service No. 105*). The \$20,500 (US) system includes the spectrofluorometer, plus a PC/AT computer with a 20 megabyte hard disk, a 640×350 inch EGA colour display and an HP ThinkJet printer. Using windows, the CRT indicates excitation and emission intensities and wavelengths, PMT high voltage, sample temperature and elapsed time, as well as derived values of ratio, total fluorescence, polarization or anisotropy. Both T-format and L-format configurations are available. Sample temperature is programmable using a Peltier heating/cooling system, and the cuvette stirrer ensures non-vortexed mixing of the sample. □

These notes are compiled by Carol Ezzell from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.